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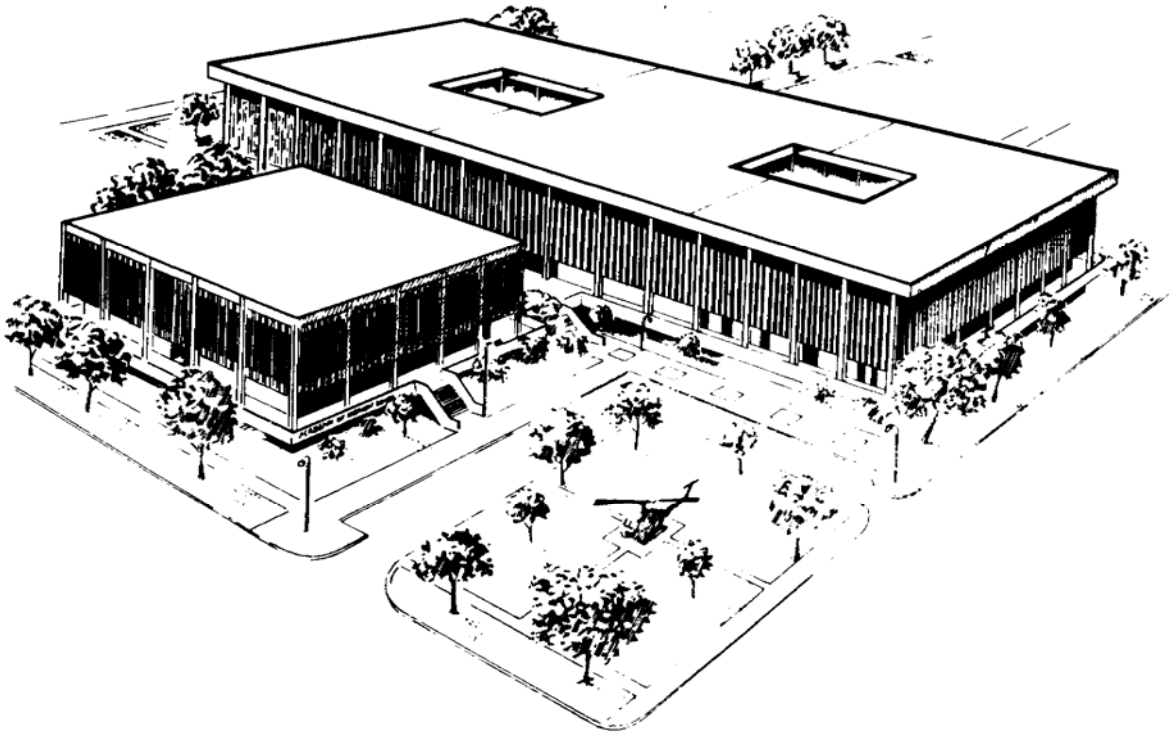
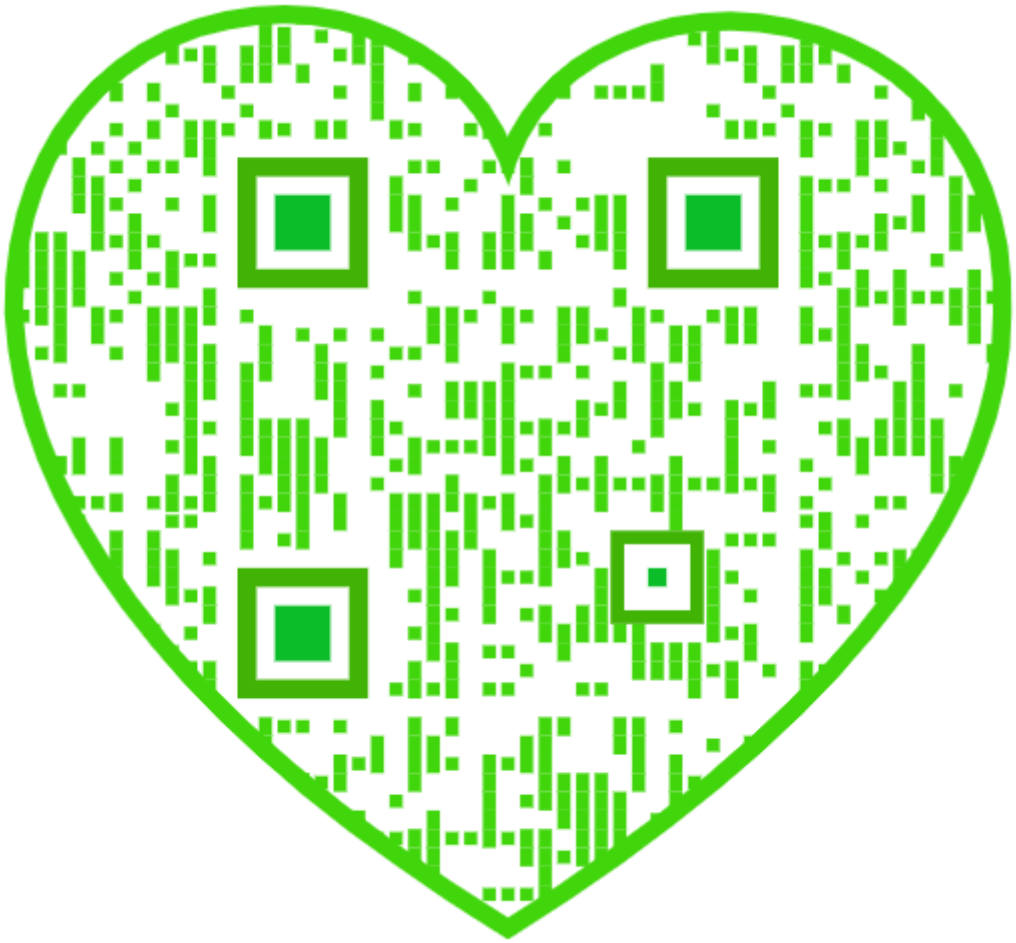


MYCOLOGY

SUBCOURSE MD0859 EDITION 200



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CLARIFICATION OF TRAINING LITERATURE TERMINOLOGY

When used in this publication, words such as "he," "him," "his," and "men" are intended to include both the masculine and feminine genders, unless specifically stated otherwise or when obvious in context.

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CORRESPONDENCE COURSE OF THE
U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL

SUBCOURSE MD0859

MYCOLOGY

INTRODUCTION

In recent years, there has been an increasing incidence of mycoses, diseases caused by fungi. This increase is largely due to the widespread use of antibiotic, immunosuppressive, corticosteroid, and cytotoxic drugs. Because of this increased incidence of mycoses, a need for laboratory specialists to learn about and to identify medically important fungi is greater than ever.

This lesson discusses important topics such as procedures for the collection and processing of mycological specimens, media and equipment, taxonomy and characteristics of important fungi, and techniques for the culture and identification of fungal pathogens.

Subcourse Components:

This subcourse consists of 11 lessons and an appendix:

Lesson 1, Introduction to Medical Mycology.

Lesson 2, Collection and Processing Procedures for Mycological Studies.

Lesson 3, Saprophytic Fungi.

Lesson 4, Yeasts of Medical Importance.

Lesson 5, Cutaneous Fungi.

Lesson 6, Subcutaneous Fungi.

Lesson 7, Systemic Mycoses.

Lesson 8, Mycological Procedures.

Lesson 9, Media and Reagents.

Lesson 10, Maintaining Stock Cultures.

Lesson 11, Commercial Kits.

Appendix, Glossary of Terms.

Here are some suggestions that may be helpful to you in completing this subcourse:

--Read and study each lesson carefully.

--Complete the subcourse lesson by lesson. After completing each lesson, work the exercises at the end of the lesson, marking your answers in this booklet.

--After completing each set of lesson exercises, compare your answers with those on the solution sheet that follows the exercises. If you have answered an exercise incorrectly, check the reference cited after the answer on the solution sheet to determine why your response was not the correct one.

Credit Awarded:

Upon successful completion of the examination for this subcourse, you will be awarded 10 credit hours.

To receive credit hours, you must be officially enrolled and complete an examination furnished by the Nonresident Instruction Branch at Fort Sam Houston, Texas.

You can enroll by going to the web site http://atrrs.army.mil and enrolling under "Self Development" (School Code 555).

LESSON ASSIGNMENT

LESSON 1	Introduction to Medical Mycology.
TEXT ASSIGNMENT	Paragraphs 1-1 through 1-7.
TASKS OBJECTIVES	After completing this lesson, you should be able to:

1-1. Select the statement that correctly describes a specific taxonomic group of fungi.

1-2. Select the statement that correctly describes the cultivation of fungi.

1-3. Identify the correct statement pertaining to specific macroscopic fungal characteristics.

1-5. Select the statement that correctly describes the microscopic characteristics of a specific fungal category.

1-6. Correctly define characteristics of different types of fungal infections.

SUGGESTION After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 1

INTRODUCTION TO MEDICAL MYCOLOGY

1-1. INTRODUCTION

Mycology is the study of fungi. A mycosis is an infection caused by a fungus. Fungi are divided into two major groups called yeasts and molds. A yeast is a unicellular, budding fungus. A mold is a multicellular, threadlike fungus. Fungi are usually slow growers, non-fastidious and are prevalent in our everyday world.

1-2. HISTORY

Mycology was one of the first microbiological sciences, beginning around 1835, when Bassi discovered that some fungi are pathogenic. In 1900, Darling characterized the pathogenic fungus, Histoplasma capsulatum. However, the majority of the techniques used today to isolate and identify fungi were developed in the early 1900's by a scientist named Sabouraud. Sabouraud is called the "Father of Mycology" because of his major contributions in mycology.

1-3. CLASSIFICATION OF FUNGI

a. Every medically important fungus belongs to one of four taxonomic groups. These groups are called zygomycetes, ascomycetes, basidiomycetes, and deuteromycetes. The different groups are distinguished by the type of spore, type of hyphae, and the type of sexual cycle.

b. Zygomycetes are characterized by aseptate or rarely septate hyphae, asexual sporangiophores, and sexual zygospores. Ascomycetes are organisms that reproduce sexually by ascospores. These fungi produce septate hyphae and several types of asexual spores. Basidiomycetes reproduce sexually by basidiospores. They also have septate hyphae. Deuteromycetes, which are also called "fungi imperfecti," have no known sexual cycle of reproduction. Many of the important human pathogens are included in this group.

1-4. CULTIVATION

a. **Yeast**. These organisms usually grow within 24 to 36 hours after inoculation on media. Routine incubation temperature is usually 25º to 30º C (room temperature).

b. **Molds**. These organisms usually grow more slowly than yeasts after inoculation to media. They are classified as rapid growers if growth occurs in less than one week. Rapid growers take more than one week for visible growth. Routine incubation temperature is usually 25º to 30º C, although 35º C incubation can be used to differentiate some molds based on temperature tolerance or to determine whether organisms are diphasic.

c. **Diphasic (Dimorphic Fungi)**. These are organisms that have two distinctly different morphologic forms that are temperature dependent. Usually this term describes fungi that grow as a mold at room temperature either as a yeast or a spherule (in tissue) at 35º C.

d. **Frequently Used Media**. The media most frequently used for the cultivation of fungi are listed below. The preparation and use of these media are discussed in Lesson 9.

(1) Sabouraud's dextrose agar (SDA).

(2) Sabouraud's dextrose agar with antibiotics (Mycosel™).

(3) Brain-heart infusion agar (BHIA).

1-5. MACROSCOPIC APPEARANCE OF FUNGI

a. The identification of fungi is based upon an evaluation of their colony characteristics as well as their microscopic morphology. Visual examination of the colony will reveal important data concerning texture, topography and pigments. Some of the textures commonly seen include:

(1) Cottony/woolly (resembling cotton candy).

(2) Suede (velvety, like pigskin).

(3) Powdery (soft, like flour or talc).

(4) Granular (gritty and more coarse than powdery).

(5) Glabrous (smooth leathery, skin-like [hairless]).

(6) Creamy (soft, buttery).

NOTE: Surface topography may be described as flat, heaped, folded, or striated.

b. Fungal colonies exhibit distinctive pigmentation on the surface and/or the reverse. Brightly colored surface pigments include blue, green, yellow, and red. Dull-colored pigments seen are brown and gray to black. Dematiaceous pigments are brown to black in color and impart a dark color to microscopic structures. Pigmentation of the reverse varies in color with some colors being characteristic of specific organisms. Water-soluble pigments are those that diffuse into the medium. Discrete pigments are those that do not diffuse into the medium.

1-6. MICROSCOPIC STRUCTURES

a. **Yeast**.

(1) General structures.

(a) Yeast cells usually grow as large, single cells. Most reproduce by an asexual process called budding. The parent cell produces a growing bud, called a blastoconidium (figure 1-1) or blastospore, which breaks away from the parent cell and grows to full size.

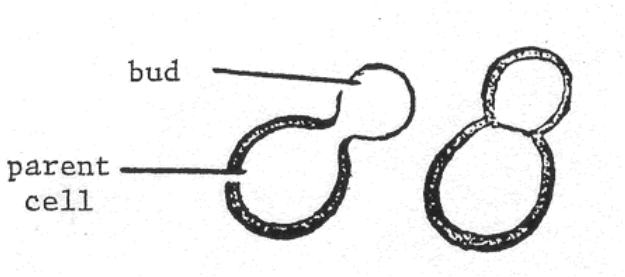


Figure 1-1. Illustration of blastoconidia.

(b) Many yeasts also produce structures called pseudohyphae (figure 1-2). These are individual blasto-conidia that have elongated and remain attached to their neighbors. These are differentiated from the "true" hyphae of molds by the "pinching" of the pseudohyphae seen at the ends of the individual segments.

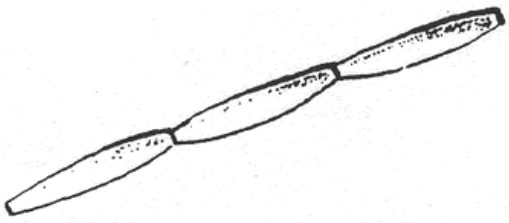


Figure 1-2. Illustration of pseudohyphae.

(c) The "true" hyphae (figure 1-3) seen in molds and certain yeasts are not pinched. Certain yeasts produce all three types of structures; blastoconidia, pseudohyphae, and hyphae.

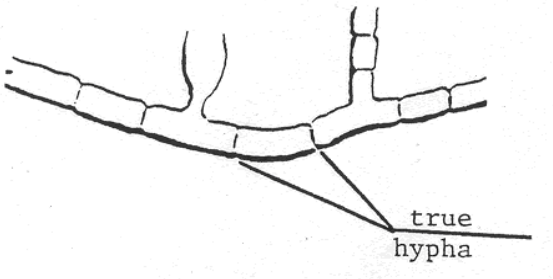


Figure 1-3. Illustration of true hyphae.

(d) Another structure produced by a few species of yeasts is the germ-tube (figure 1-4). It is a tubal-like structure that grows from the surface of the yeast cell. It has parallel, non-constricted walls. Germ tubes are seen in direct examination of specimens or following growth of yeast cells in media that stimulates their production.

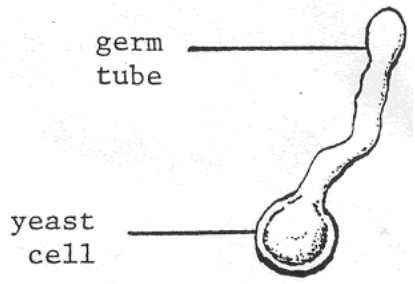


Figure 1-4. Illustration of a germ tube.

(2) Sexual reproduction. Yeasts reproduce sexually by either ascospores or basidiospores. Ascospores are sexual spores formed within a special sac, called an ascus. Basidiospores are formed on top of a club-shaped structure, called a basidium.

(3) Asexual reproduction. All true yeasts must produce blastoconidia and may produce other asexual structures as well. Yeasts may produce pseudohyphae (elongated blastoconidia) and "true" hyphae. If the "true" hyphae fragment into individual cells, these cells are called arthroconidia. Most yeasts produce blastoconidia and pseudohyphae. An exception is Trichosporon beigelli, which produces blastoconidia, arthroconidia, pseudohyphae, and true hyphae.

b. **Molds**.

(1) General structures. The individual filament of a mold is called a hypha (plural hyphae). The walls of the hyphae are parallel and are not constricted, as the pseudohyphae are that are seen with yeasts. There are three types of hyphae: (1) vegetative hyphae, which absorb the nutrients from the culture medium; (2) aerial hyphae, which grow above the agar and give the colony its texture; and (3) fertile hyphae, which produce the reproductive structures. Large collections of hyphae are given the term mycelia. Hyphae may or may not produce cross-walls, called septations. These septations are formed in a centripetal manner, meaning they start from each of the parallel walls and meet in the center, completing the formation of the cross-wall.

(2) Sexual reproduction. Molds reproduce sexually via ascospores or basidiospores (as discussed above), or by zygospores. Zygospores are formed following the contact and union of two hyphal tips. These hyphal tips contain sexual spores and join together to form a thick-walled resting spore called the zygospore. This spore is larger than the hyphae and is formed by the flow of protoplasm and nuclei into the structure.

(3) Asexual reproduction.

(a) Molds reproduce asexually using a variety of cells or structures. In the class of Zygomycetes, the basic structure is called a sporangium (figure 1-5) pl. sporangia. It is a saclike cell in which the internal structures are cleaved into individual units, called sporangiospores. These sporangiospores may be randomly distributed in the sporangium, or may be arranged in a row. The sporangia are supported by a specialized hypha called a sporangiophore that arises from the vegetative hyphae.

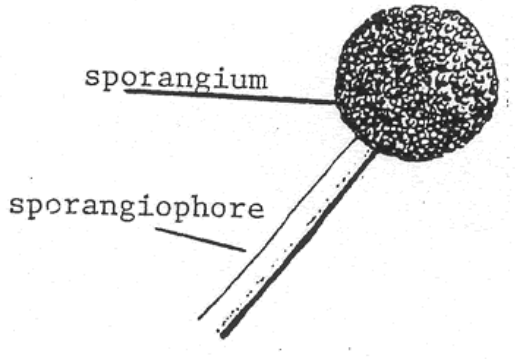


Figure 1-5. Illustration of sporangium and sporangiophore.

(b) Most fungi of medical interest do not produce sporangia, but produce structures called conidia (figure 1-6). Conidia, by definition, are structures that develop by asexual means other than by production of a sporangium. Conidia are produced from specialized hyphae called conidiogenous cells (conidiophores). When these cells are large and multicellular, they are called macroconidia. When they are small and unicellular, they are called microconidia.

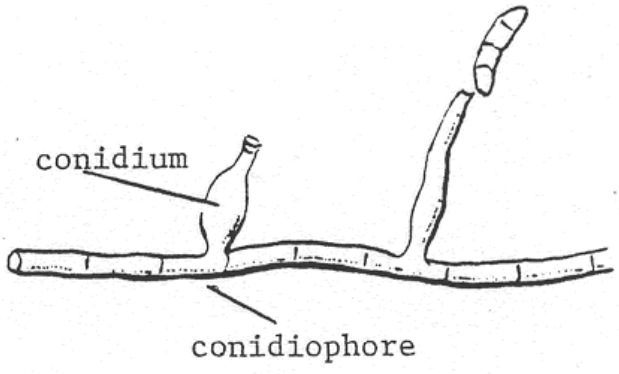


Figure 1-6. Illustration of conidia and conidiophore.

c. **Types of Conidia**. Types of conidia are based on their cells of origin (table 1-1).

(1) Aleurosconidia: conidia produced singly as "blown-out" ends of conidiogenous cells.

(2) Annelloconidia: blastoconidia, produced at the base of the conidiogenous cell (annellide), the oldest conidium is at the apex with the youngest at the base. As each conidium is produced, a scar or annellation is left at the outer surface of the conidiogenous cell.

(3) Arthroconidia: conidia produced by fragmentation of hyphae into individual cells.

(4) Blastoconidia: conidia produced as buds or outgrowths from a portion of the pre-existing or parent cell.

(5) Phialoconidia: conidia produced from a conidiogenous cell (phialide) that does not increase in length as conidia are produced.

(6) Poroconidia: conidia produced through pores in the conidiagenous cell.

MOLDS	
Sexual Structures	**Asexual Structures**
Ascospores Basidospores Zygospores	Sporangiospores Conidia Aleuroconidia Annelloconidia Blastocondia (buds) Phialocondia Porocondia
YEAST	
Sexual Structures	**Asexual Structures**
Ascospores Basidospores	Blastocondia (buds) Pseudohyphae Arthrocondia (hyphae)

Table 1-1. Sexual and asexual structures produced by fungi of medical importance.

1-7. MYCOSES

Diseases caused by fungi are collectively called mycoses. They are divided into four general categories on the basis of the primary tissue affinity of the pathogen.

a. Superficial mycoses are infections limited to the hair and dead layers of the skin (stratum corneum).

b. Cutaneous mycoses (dermatophytoses or ringworm) affect only the skin, hair, and nails.

c. Subcutaneous mycoses affect the subcutaneous tissue below the skin, and occasionally, the bone.

d. Systemic mycoses infect the internal organs and may spread throughout the host. They are the most serious of the mycoses.

Continue with Exercises

EXERCISES, LESSON 1

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. The individual considered to be the father of mycology is:

   a. Bassi.

   b. Darling.

   c. MacFarland.

   d. Sabouraud.

2. Which of the following is used to distinguish the taxonomic group of a fungus?

   a. Sexual cycle.

   b. Hyphal morphology.

   c. Spore type.

   d. All of the above.

3. What is the usual incubation temperature for yeast cultivation?

   a. 18º - 20º C.

   b. 20º - 30º C.

   c. 25º - 30º C.

   d. 30º - 40º C.

4. Fungi that can grow either as a mold or yeast are:

    a. Birmorphic.

    b. Diphasic.

    c. Rarely isolated.

    d. Grown at 20º C.

5. Macroscopic characteristics used to identify fungi include texture, pigment, and:

    a. Type of hyphae.

    b. Spore structure.

    c. Topography.

    d. All the above.

6. One of the sexual reproductive structures in yeasts is:

    a. Blastoconidium.

    b. Basidiospore.

    c. Chlamydospore.

    d. Germ tube.

7. If a fungal colony is described as glabrous, its texture should appear to be:

    a. Soft and buttery.

    b. Smooth and leathery.

    c. Soft and floury.

    d. Gritty and coarse.

8. An individual filament of a mold is a:

   a. Hypha.

   b. Mycelium

   c. Yeast cell.

   d. Germ tube.

9. Hyphae may be described as:

   a. Vegetative.

   b. Aerial.

   c. Fertile.

   d. All the above.

10. What are the four categories of mycoses?

   a. _____

   b. _____

   c. _____

   d. _____

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 1

1. d. (para 1-2)

2. d. (para 1-3a)

3. c. (para 1-4a)

4. b. (para 1-4c)

5. c. (para 1-5a)

6. b. (para 1-6a(2))

7. b. (para 1-5a(5))

8. a. (para 1-6b(1))

9. d. (para 1-6b(1))

10. Superficial.
    Cutaneous.
    Subcutaneous.
    Systemic. para 1-7).

End of Lesson 1

LESSON ASSIGNMENT

LESSON 2	Collection and Processing Procedures for Mycological Studies.
TEXT ASSIGNMENT	Paragraphs 2-1 through 2-15.
TASKS OBJECTIVES	After completing this lesson, you should be able to:

2-1. Select the statement that correctly describes procedures for collecting a specific type of specimen for mycologic study.

2-2. Select the statement that correctly describes procedures for processing a specific type of specimen for mycologic study.

2-3. Select the statement that correctly describes procedures for examining a specific type of specimen for mycologic study.

SUGGESTION After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 2

COLLECTION AND PROCESSING PROCEDURES FOR MYCOLOGICAL STUDIES

2-1. INTRODUCTION

The principal goals of a clinical mycology laboratory are to isolate and accurately identify pathogenic fungi. Proper collection, transport, processing, and culturing of clinical mycology specimens is essential to achieve these goals. Laboratory personnel must be knowledgeable about specimen requirements if optimal results are to be obtained from the study of clinical specimens. This lesson covers information and procedures necessary for processing specimens for mycological studies.

2-2. ABSCESS SPECIMENS

a. **Collection**. These specimens are collected from nondraining abscesses by aseptically aspirating the material with a sterile needle and syringe. Pus from miliary abscesses (abscesses, resembling millet seeds) is collected by opening the abscess with a sterile scalpel and expressing the material into sterile tubes. Swabs should NOT be used to collect specimens for culture of fungi.

b. **Processing**. Process abscess material within two hours of collection. Refrigerate specimens prior to processing if delay is unavoidable.

(1) Direct examination. Prepare specimen for direct examination using 10 percent KOH and/or periodic acid-Schiff stain (PAS). Fungal morphology can be observed using either a bright-field or phase-contrast microscope.

(2) Inoculation. Inoculate material directly onto isolation media. If a large volume of specimen is available, centrifuge for 15 minutes at 2500 RPM and process the sediment. Media to be used for inoculation are Sabouraud dextrose agar (SDA), Mycosel™ or Mycobiotic™ agar (containing cycloheximide and chloramphenicol), and brain heart infusion agar (BHIA).

(3) Incubation. Incubate inoculated media at 30º C and examine for growth every two to three days. Identify all recovered fungi. Discard cultures as negative for fungi after four weeks of incubation.

2-3. BIOPSY MATERIAL

a. **General**. With the exception of a liver biopsy, tissue containing a portion of the wall, base, and center of the lesion should be obtained for mycotic studies. The specimen should be placed between sterile moist gauze squares, placed in a sterile container or petri dish, and sent to the laboratory immediately. Liver biopsies usually yield a small core of tissue that must be shared by the microbiology and pathology laboratories. It is recommended that the portion to be subjected to microbiologic studies be placed directly into a tube of brain heart infusion broth or Sabhi broth and sent to the laboratory. A sterile glass rod should be used to crush the soft tissue in the broth. Microscopic studies will be made by the pathologist. If lymph nodes are biopsied, a portion of several nodes should be sent to the microbiology section.

b. **Processing of Tissue for Mold or Yeast Isolation**.

(1) Examine for presence of purulent, caseous, or necrotic areas. Such material should be inoculated directly to media and at least five thin smears made on glass slides (not exceeding 1.0 mm in thickness). These smears may be subjected to various stains including the modified PAS stain. If indicated, one or more of these smears may be used for fluorescent antibody studies. A KOH preparation should also be examined. If indicated, an India ink preparation may also be examined.

(2) Cut tissue into small fragments with sterile scissors and forceps.

(3) Place tissue fragments into a 15-mL homogenizer, or into a mortar, and thoroughly homogenize with pestle. Care should be taken to avoid any aerosol resulting from grinding the specimen.

(4) Two to 3 mL of Sabouraud or BHI broth may be added to the tissue as homogenization progresses.

(5) Pipette 0.5 mL of the homogenate to each plate of medium to be used. The remaining homogenate should be inoculated to BHIB.

c. **Processing Tissue for Mycetomas**.

(1) A mycetoma is a tissue tumor composed of granules. There are two basic types of mycetomas: actinomycolic and eumolytic. The actinomycotic mycetomas are caused by Nocardia species, Actinomyces species, or Streptomyces species of bacteria. The crushed granules of an actinomycotic mycetoma will show microscopic coccid and bacillary forms. These organisms can also produce filaments that are easily confused with hyphae or mycelia. The crushed granules of eumycotic mycetomas microscopically will show hyphae and/or mycelia because the organisms causing the mycetomas are molds.

NOTE: Other bacteria, such as Escherichia coli, Staphylococcus aureus, Proteus spp, and Pseudomonas aeruginosa may cause a similar disease called botryomycosis, which also gives rise to granules. Therefore, presence of a "granule" doesn't confirm that an actinomycotic mycetoma is present.

(2) In some instances, the organisms within a granule may be dead or dying and will not grow when inoculated to culture media. For this reason careful microscopic studies must be made of one or more granules to determine whether an actinomycete, some other bacterium, or a mold, is the causative agent. The agent present determines therapy and prognosis.

(3) Examination of tissue for presence of granules.

(a) Place tissue in the bottom of a sterile petri dish.

(b) If granules are not visible through a hand lens, place the petri dish under a dissecting scope. With sterile teasing needles, gently but quickly dissect the tissue while looking through the dissecting scope.

(c) If granules are absent, streak any purulent and/or necrotic tissue directly to SDA, Mycosel™ , and BHIA.

(4) Microscopic examination of a granule.

(a) Place the granule in a drop of water (do not use KOH since it may be necessary to do a Gram stain or a PAS stain on the material).

(b) Gently tease the granule apart.

(c) Place a coverslip over the preparation and examine microscopically. An actinomycotic granule will usually reveal beaded branching filaments that are approximately 1.5 to 2.0 microns in diameter. It is not possible to differentiate an aerobic actinomycete from an anaerobic strain by microscopic study of the granule. Cultures are necessary to confirm the identity of the organism. A eumycotic granule will contain hyphae that measure 4 to 7 microns in diameter. Swollen cells, suggestive of chlamydospores, may also be observed. Chlamydospores are thick-walled cells formed by the rounding-up of a cell. They may or may not germinate to form additional hyphae.

NOTE: Granules seen in botryomycosis may reveal filamentous, nonbranching bacilli, often in association with cocci.

(5) Preparation of granules for inoculation of media.

(a) Tease several granules free from the specimen and place them in a small test tube containing 2 to 3 mL of sterile, distilled water. Gently shake the tube and then transfer the washed granules with a pipette to a second tube of sterile, distilled water. Shake the second tube and content.

(b) Transfer the washed granules to a small centrifuge tube containing 0.5 to 1.0 mL of sterile, distilled water. Crush the granules with a glass rod. Inoculate the suspension using a sterile pipette. Streak agar plates with a loop. Inoculate broth media with several drops of the suspension.

2-4. BLOOD CULTURES

a. The compromised host is very susceptible to mycotic infections. Candidiasis is the most common of the deep-seated fungal infections in these patients. Even when systemic candidiasis is suspected, only a small percentage of the cases are diagnosed before death. Thus, the physician and the laboratorian are faced with two major tasks. The first is to employ the most reliable method for isolation of yeast from blood as quickly as possible. The second is to obtain data that will assist in differentiating catheter associated fungemia from other forms of fungemia.

b. When attempting to differentiate catheter-associated fungemia from other septic episodes, the following specimens are to be collected within the same time period: (1) a blood culture collected from the arm not containing the catheter, (2) a blood culture drawn through the catheter, (3) culture of the catheter tip, and (4) additional blood cultures over a 24 to 48 hour period, all collected from the arm that did not have the catheter. Smears of the blood and material from the catheter tip should be stained immediately with either the PAS or Wright's stain. If any exudate is present at the insertion site of the catheter tip, this should also be stained and examined. Regardless of the method used for culturing blood, sodium polyanethol sulfonate (SPS, Liquoid) should be used as an anticoagulant. Sodium polyanethol sulfonate is the least toxic of the anticoagulants and inactivates complement and leukocytes. The ratio of blood to broth in a culture should be 1:10 to 1:20. A higher concentration of blood will markedly reduce the chance of isolating an organism.

c. Prepare the skin for venipuncture.

(1) Clean the skin thoroughly with 70 percent ethyl or isopropyl alcohol at the site where venipuncture is to be made.

(2) If the patient is not allergic to iodine, swab the site concentrically with 2 percent tincture of iodine. Remove the iodine with an alcohol swab when venipuncture is completed.

d. Use the standard technique for blood culture.

(1) As outlined above, collect the blood in a Vacutainer tube or inoculate it directly to a biphasic blood culture bottle containing SPS. Biphasic blood culture bottles contain an agar slant to help isolate the disease-causing organism. Remember to maintain a ratio of 1:10 to 1:20 of blood to broth.

(2) Immediately and gently mix the contents of the bottle to prevent clotting and to wash the surface of the agar slant. If a biphasic blood culture bottle is not used, the blood-broth mixture must still be mixed.

(3) If blood was collected in a Vacutainer tube, it may be transferred to one or more blood culture bottles. Use of a transfer set greatly reduces the chance of contamination.

(4) Vent all blood culture bottles. Fungi will not survive under anaerobic conditions. Incubate at room temperature.

(5) If biphasic bottles are used, examine the agar slant daily for presence of fungal colonies. If no colonies are seen and broth is clear, gently "wash" the agar surface with the blood-broth mixture and reincubate. Culture media should be held at least 4 weeks before reporting as negative for fungi.

(6) If only bottles containing a broth are used, examine daily for gross evidence of growth. Subculture the broth every 48 hours. Blood cultures should be held for 4 weeks. Candida guilliermondii and Torulopsis glabrata may require 28 to 30 days to grow in routine blood culture bottles. Discard bottles as negative after four weeks.

2-5. BONE MARROW

a. This specimen is useful in the diagnosis of histoplasmosis, disseminated candidiasis, and cryptococcosis.

b. Procedure.

(1) Approximately 0.25 to 0.3 mL of bone marrow is collected in a heparinized syringe.

(2) A sterile cap is placed over the syringe and is sent immediately to the laboratory.

(3) The specimen is inoculated to several plates of HIA or Sabhi agar.

(4) Several fragments of marrow are used to prepare smears for PAS or Giemsa stains.

(5) Following inoculation of agar plates with the marrow specimen the syringe is ringed aseptically with sterile BHIB and the rinse is then incubated.

(6) All culture media are incubated at room temperature and are held at least 4 weeks before being discarded. If Histoplasma capsulatum is suspected, the cultures are incubated for 10 to 12 weeks before discarding as negative.

(7) If the specimen contains a large quantity of blood, it is centrifuged and the supernatant is removed with a sterile pipette and placed in a blood culture medium. The sediment is then inoculated to plates as outlined above.

2-6. GENERAL SPINAL FLUID

a. At least 3 mL are needed for adequate mycologic studies. Use of the membrane filter technique will permit culturing for Cryptococcus neoformans, Candida albicans, and Mycobacterium tuberculosis simultaneously. If this technique is not used, it will not be possible to setup satisfactory cultures for fungi and Mycobacterium tuberculosis on the same spinal fluid sample.

b. Membrane filter technique.

(1) Centrifuge specimen at 2000 rpm for 15 minutes.

(2) Do not decant supernatant. Using a capillary pipette, remove a small portion of the sediment and make an India ink preparation and at least two thin smears that may be used for PAS, Acid Fast, or Gram stains.

(3) Thoroughly mix the specimen on a mechanical mixer and pass the entire sample through a 0.45 micron membrane filter.

(4) Place the membrane (inoculated surface up) on BHIA or Sabhi agar plates.

(5) Seal each plate with parafilm or place in a plastic bag and incubate at 35º C for two weeks before discarding as negative.

(6) All membrane filter cultures should be examined every 24 to 48 hours under a dissecting scope (leave top on Petri dish). Growth of yeast may be first observed as a "film" on the membrane surface, developing into actual colonies in another 24 to 48 hours. When a "film" is evident, sweep the membrane surface with a sterile loop and wash the loop in a drop of water onto a clean glass slide. Cover the drop with a coverslip and examine the preparation for presence of yeasts or bacteria. If desire, the coverslip may be removed and the slide allowed to air dry for Acid Fast or Gram stains to be done. Subculture any organism preset to SDA, Mycosel™, and BHIA.

2-7. HAIR, SKIN, AND NAILS (DERMATOPHYTES)

a. **Hair**.

(1) The patient's scalp should be examined with Wood's lamp (ultraviolet light) for the presence of fluorescing hairs. No cleaning of the scalp is needed.

(2) Using forceps, epilate (luck) at least 10 to 20 fluorescent or broken hairs and place them between clean glass slides, or in a clean pill envelope sealed with cellophane tape. Wrap slides in a piece of paper. On this paper or envelope, write the patient's data, including notation of therapy received before epilation of hair.

NOTE: Hairs that break off at the scalp level when using forceps may have been invaded by T. tonsurans and are best epilated with a knife blade. Scraping the scalp rarely yields hairs that have been invaded.

(3) Processing.

(a) Using KOH or PAS, mount the specimen for direct microscopic examination. Observe specimen for fungal elements and for ectothrix or endothrix hair invasion.

(b). Place three or four hairs each on SDA and Mycosel™ media.

I (c) Incubate at 30º C for four weeks before discarding as negative.

(d) Examine every two days for growth. Identify all fungi.

b. **Skin and Nails**.

(1) Glabrous skin.

(a) Wipe lesion(s) well with an alcohol gauze sponge. Cotton balls leave too many fibers on the skin that may interfere with interpretation of the direct examination. Instead of alcohol, use sterile water or broth if the area is irritated or the skin is broken.

(b) Scrape the entire periphery of a lesion(s) with a sterile scalpel (if more than one lesion is present, several should be scraped). Place scrapings in an envelope or between clean glass slides as discussed in paragraph 2-7(a)(2).

NOTE: Lesions should be untreated with topical antifungal agents for at least one week if isolation of fungi is to be successful.

(2) Interspaces (between toes).

(a) Clean interspaces with a gauze square moistened with 70 percent alcohol or sterile water. Remove all dried exudate.

I (b) Using a scalpel, gently scrape both sides and base of each interspace. If fissuring is present, scrape only the sides.

NOTE: The fourth interspace of each foot should always be included when collecting specimens for a laboratory diagnosis of tinea pedis. A dermatophyte may be present in this area in patients with skin reactions or with asymptomatic infections.

(3) Nails.

(a) Clean nails with a gauze square.

(b) Remove a portion of debris from under the nail with a scalpel and place between clean glass slides.

(c) If the dorsal plate appears diseased, scrape the outer surface. It is recommended that the first four or five scrapings be discarded. This will help eliminate contaminating spores and bacteria. Collect scrapings through the diseased portion.

(d) An evulsed nail should be placed in a petri dish or envelope.

(e) Nail clippings for isolation of dermatophytes require micronizing the nails before inoculation to media.

(4) Skin.

(a) Lesions due to Candida species. Obtain scrapings from any portion of the lesion (center or edge) and place on a clean glass slide. If scrapings are moist, place a "match stick" or a small piece of folded paper between the bottom slide (containing scrapings) and the slide used to cover the specimen. This will prevent the two slides from "freezing" together when the scrapings dry.

(b) Lesions due to Malassezia furfur. Collect scrapings from more than one lesion. The entire lesion may be scraped.

(c) Skin biopsy specimen. Place the specimen between two moistened gauze squares. Place these in a petri dish. This permits microscopic examination of the specimen for white blood cells (pus). If the specimen is collected by punch biopsy, place it in a small tube of sterile water or sterile saline. Process the specimen in the laboratory immediately.

(5) Processing.

(a) Place the specimen fragments into a drop of 10 percent KOH and then cover with a cover glass. Pass the slide several times through a flame. DO NOT OVERHEAT OR BOIL! Examine preparation by bright-field or phase-contrast microscopy. The more sensitive PAS stain may be used if fungi are suspected but not observed in the KOH preparation.

(b) Inoculate portions of the specimen on SDA and Mycosel™ media.

(c) Incubate at 30º C and examine every two or three days. Discard negative cultures after four weeks.

(d) Identify all fungi.

2-8. DRAINING FISTULA, SINUS TRACT

a. Swabs used to collect material from these lesions rarely yield fungi. The specimen should be obtained by curettage from deep in the lesion and should include part of the wall. Such specimens are more likely to contain granules or fungal elements.

b. The specimen should be placed in a sterile tube containing 2 to 3 mL of sterile water or saline.

c. Occasionally, if the lesion is due to an actinomycete, granules may be sloughed out into the tract. Flushing the tract with sterile saline may yield these granules. These washings should be collected in a sterile test tube. Covering the tract opening with a gauze pad for 12 to 24 hours may permit entrapment of granules in the pad. Bacterial contamination of a specimen collected in this manner may suppress the growth of actinomycetes or molds. However, the morphology of organisms in these granules can usually be determined.

d. Both the washings and gauze pad should be examined with a hand lens for presence of granules. Granules should be removed with either a sterile capillary pipette or forceps and processed as outlined under "Biopsic Material."

2-9. JOINT FLUID

This specimen should be collected in a sterile tube containing either heparin or SPS to prevent clotting.

a. Centrifuge the specimen at 2500 rpm for approximately 30 minutes.

b. With sterile pipette, place the supernatant into a tube of BHI broth.

c. Inoculate 0.1 mL of the sediment to two plates each of SDA, Mycosel™, and BHIA.

d. Incubate one set of plates at room temperature and the second set at 35º C. Hold all cultures at least four weeks before discarding as negative.

2-10. NASOPHARYNGEAL (NP)

Nasopharyngeal specimens are not routinely cultured for fungi and are only one of several specimen types that may be collected when a diagnosis of systemic or disseminated aspergillosis or candidiasis is suspected.

a. Place the NP swab in a sterile dry tube or a tube containing 2 to 3 mL of Sabouraud broth. Do not use transport media. Specimens should reach the laboratory within 30 minutes.

b. Streak the swab over a SDA plate and a Mycosel™ plate.

c. If the swab is received in broth, incubate the broth with culture plates at room temperature.

2-11. PLEURAL FLUID

a. The specimen should be collected with heparin to prevent clotting.

b. If the specimen is not purulent, centrifuge at 2500 rpm 15 minutes.

c. Decant and inoculate 0.1 mL of the sediment to SDA, Mycosel^{tm}, and BHIA.

d. Make a KOH preparation and several thin smears of the sediment.

e. Incubate the plates and remainder of specimen at room temperature for at least four weeks.

NOTE: If the specimen is purulent, it is processed and inoculated to media in the same manner as outlined for sputum and bronchial washings. If culturing for Nocardia is requested, inoculate media to isolate these organisms before adding chloramphenicol to the sediment.

2-12. SPUTUM BRONCHIAL WASHINGS

a. One of the greatest challenges to the clinician and the microbiologist is diagnosis of pulmonary disease seen in the compromised host. The need for teamwork between the clinician and microbiologist is crucial. A sputum specimen must represent material from the lungs. Saliva and nasal secretions are unsatisfactory. An early morning specimen that is likely to contain secretions from the tracheobronchial tree is desirable. Collect one specimen per day for three days.

b. Sputum should be collected and then placed in a sterile screw cap container. Bronchial washings are usually placed in a sterile tube. The specimen should be examined carefully for flecks containing pus and caseous or bloody material. Such flecks are most likely to contain fungal elements. They should be plated and smears made from them. If careful examination of the specimen does not reveal such flecks, it should be concentrated, since there may be only a few fungal cells in sputum or bronchial washings.

c. Processing. Sputum specimens should be processed within two to four hours. If processing must be delayed, refrigerate the specimen.

(1) Using 10 percent KOH and/or PAS, mount the specimen for microscopic examination. Observe the specimen for fungal elements using bright-field or phase-contrast microscopy.

(2) The sediment of concentrated specimens should be inoculated to plates (or bottles) of media rather than to agar slants. Surface area is of great importance in attempting to isolate fungi from contaminated material.

(a) Concentration of specimen for isolation of yeasts and molds.

1 Place equal volumes of digestant (N-acetyl-l-cysteine or Dithiothreital without NaOH) and the specimen in a 50-mL graduated centrifuge tube, preferably with a screw cap.

2 Mix on a Vortex mixer for 5 to 10 seconds. Mixing time may be extended if sputum is tenacious.

3 Add enough M/15 phosphate buffer solution (pH 6.8 - 7.1) to bring the volume up to 500 mL.

4 Centrifuge at 2500 rpm for 15 minutes.

5 Decant supernatant.

6 Add enough chloramphenicol to the sediment to give final concentration of 0.05--0.1 mg/mL. If Histoplasma capsulatum is suspected, substitute penicillin (20 units/mL) or streptomycin (40 mg/mL) because the yeast phase of H. capsulatum is susceptible to chloramphenicol.

7 Mix thoroughly and place 0.1 mL of the sediment on each plate (or in each bottle) of medium.

8 Make a KOH preparation of a drop of the sediment; make three or four thin smears, and allow to air dry. These may be used for PAS, Giemsa, Acid Fast, or Gram stains.

(b) Concentration of sputum for isolation of Nocardia species.

1 Concentrate as outlined above.

2 Do not add chloramphenicol to sediment.

3 Inoculate plates as for mold isolation; thin smears should also be made. Cultures of molds, yeasts, and Nocardia species can be made using one concentrated specimen, if a portion of the sediment is inoculated to 7H10 medium before chloramphenicol is added.

(3) Inoculate portions of the specimens on SOA, Mycosel™ yeast extract phosphate plate.

(4) Incubate at 30º C and examine daily. Discard negative cultures after four weeks. If Histoplasma is suspected, discard negative cultures after 12 weeks.

(5) Identify all fungi.

2-13. THRUSH LESIONS OF ORAL CAVITY

a. Split a tongue depressor in half along its long axis.

b. Use one half the tongue depressor to gently scrape the lesion.

c. Insert the depressor into a sterile test tube and send to the laboratory immediately.

d. Tease a portion of the material on the stick, into a drop of KOH on a clean glass slide.

e. Mount with a cover slip and examine for presence of pseudohyphae and blastoconidia.

f. Scrape the remainder of the specimen onto the agar surface of a plate of Sabouraud dextrose agar containing only chloramphenicol. With a sterile loop, spread the inoculum over the medium surface.

g. Incubate the plate at 30º C for one week before discarding.

NOTE: Material from thrush lesions should not be collected with swabs. The fungus is usually firmly attached to the mucous membranes and only blastoconidia may be obtained using the swab. The laboratory diagnosis of thrush requires the demonstration of pseudohyphae as well as blastoconidia.

2-14. URINE SPECIMEN

a. This specimen may yield C. neoformans in a patient with cryptococcosis before the organism is revealed in sputum or spinal fluid. It also may be a valuable specimen when testing a patient for disseminated candidiasis or candidiasis of the urinary tract. Infections of the upper bladder or upper urinary tract may sometimes require urine specimens collected from each kidney by means of a cystoscope. Before collecting specimens, the bladder should be flushed to reduce chances of contaminating specimens obtained from the kidneys with yeasts that may be in the bladder. The three specimens (one from each kidney and one from the bladder) should be clearly labeled. The laboratorian must be careful not to mix them during processing.

b. A urine specimen collected by percutaneous needle biopsy reduces the problem of contamination with organisms present in the lower urethra or external genitalia. A clean catch midstream specimen should be collected when percutaneous bladder aspiration and cystoscopy are thought to be unnecessary by the physician.

c. Process urine within two to four hours *of* collection. Refrigerate specimens if a delay in processing is unavoidable.

(1) Centrifuge urine at 2000 rpm for 10 to 15 minutes. Decant the supernatant.

(2) Direct examination. Prepare the specimen for direct examination by mixing a drop of sediment with one percent KOH and/or PAS on a clean glass slide, then coverslip. Observe for fungal elements.

(3) Place 0.05 to 0.1 mL of the sediment on each plate of SDA, Mycosel™, and BHIA.

(4) Incubate at 30º C for 2 weeks before discarding as negative.

(5) Examine every 2 to 3 days for growth. Identify all fungi.

2-15. VAGINAL

a. Several swabs containing material from the vagina should be inserted into tubes of Sabouraud broth and sent to the laboratory immediately. A transport medium is not recommended.

NOTE: Although Candida albicans will survive in most transport media, insufficient data is available concerning the survival of other fungi in these media. When transport media are used, an adequate specimen adhering to the swab is usually not available for KOH preparations.

b. Tease material from a swab directly into a drop of KOH on a clean glass slide. Mount with a coverslip and examine the preparation carefully for any fungal elements.

c. Use a second swab to streak a plate of Sabouraud agar containing chloramphenicol.

d. Incubate plates at 25º C for four to five days before discarding as negative.

Continue with Exercises

EXERCISES, LESSON 2

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Direct examination of smears for fungal morphology is done using:

   a. A monocular microscope.

   b. Phase-contrast microscopy.

   c. Smears made from culture growth.

   d. Gram stain.

2. Media used to isolate fungi from abscess specimens include:

   a. Mycosel™.

   b. Sabouraud dextrose agar.

   c. Brain heart infusion agar.

   d. All of the above.

3. Mycology specimens obtained from a liver biopsy usually include:

   a. Portions of several nodes.

   b. Portions of the wall, base, and center of the lesion.

   c. A single small tissue core.

   d. Purulent material.

4. The presence of "granules" in biopsy tissue confirms the presence of a Nocardia species infection.

    a. True.

    b. False.

5. Microscopic examination of an actinomycotic granule shows:

    a. Hyphae 4-7 mcm in diameter.

    b. Beaded branching filaments.

    c. Filamentous nonbranching bacilli.

    d. Differences between aerobic and anaerobic actinomycetes.

6. Collection of blood for the detection of fungemia requires the use of _____ as an anticoagulant.

    a. EDTA.

    b. Sodium citrate.

    c. Citrate-phosphate-dextrose.

    d. Sodium polyanethol sulfonate (SPS).

7. The most commonly found fungal infection is:

    a. Candidiasis.

    b. Histoplasmosis.

    c. Tuberculosis.

    d. Botryomycosis.

8. Blood culture bottles for fungal growth must be:

    a. Incubated at 30º C.

    b. Incubated under anaerobic conditions.

    c. Examined every two or three days for growth.

    d. Vented to create aerobic conditions.

9. The syringe used to collect bone marrow for fungal study should be:

    a. Coated with heparin before specimen collection.

    b. Rinsed with BHIB and the rinse fluid incubated.

    c. Capped for transportation.

    d. All of the above.

10. Use of the membrane filter technique for cerebral spinal fluid permits the:

    a. Use of the Sabhi agar culture medium.

    b. Simultaneous culture of fungi and H. tuberculosis.

    c. Identification of C. neoformans.

    d. Subculturing of early growth.

11. Specimens of hair, for isolation of fungi, should be cultured on:

    a. BHIB medium.

    b. Mycosel™ medium.

    c. KOH medium.

    d. Sabhi broth.

12. Collection of a specimen from glabrous skin is done by:

    a. Flushing the infected area with sterile water.

    b. Covering an open lesion with a gauze pad for 12 to 24 hours.

    c. Scraping the periphery of a lesion with a sterile scalpel

    d. Removing loose cells with sterile forceps.

13. The best method for collecting a specimen for fungal studies from a draining fistula is the use of a swab.

    a. True.

    b. False.

14. Joint fluid specimens are inoculated on/into:

    a. Sabouraud broth.

    b. BHI broth.

    c. A membrane filter.

    d. Blood culture medium.

15. Flecks which may be seen in sputum specimens are likely to:

    a. Contain fungal elements.

    b. Indicate bacterial infection.

    c. Interfere with mycology studies.

    d. Indicate specimen contamination.

16. How long must culture plates be retained if Histoplasma is suspected?

    a. Two weeks.

    b. Four weeks.

    c. Eight weeks.

    d. Twelve weeks.

17. Laboratory diagnosis of thrush:

    a. Is usually made from the culture of a sputum specimen.

    b. Depends on the demonstration of blastoconidia.

    c. Depends on the demonstration of pseudohyphae.

    d. Requires the demonstration of blastoconidia and pseudohyphae.

18. Culture of a urine specimen:

    a. Is helpful in looking for candidiasis of the urinary tract.

    b. May yield C. neoformans.

    c. Is helpful in looking for disseminated candidiasis.

    d. All of the above.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 2

1 b (para 2-2b(1))

2. d (para 2-2b(2))

3. c (para 2-3a)

4. b (para 2-3c NOTE)

5. b (para 2-3c(4)(c))

6. d (para 2-4b)

7. a (para 2-4a)

8. d (para 2-4d(4))

9. d (para 2-5b)

10. b (para 2-6a)

11. b (para 2-7a(3)(b))

12. c (para 2-7b(1)(b))

13. b (para 2-8(a))

14. b (para 2-9b)

15. a (para 2-12b)

16. d (para 2-12c(4))

17. d (para 2-13 NOTE)

18. d (para 2-14a)

End of Lesson 2

LESSON ASSIGNMENT

LESSON 3	Saprophytic Fungi
TEXT ASSIGMMENT	Paragraphs 3-1 through 3-21.
LESSON OBJECTIVES	Upon completion of this lesson, you should be able to:

3-1. Select the statement that correctly describes the importance of saprophytic fungi.

3-2. List the disease characteristics that describe aspergillosis.

3-3. List the characteristics of a specific zygomycete.

3-4. List characteristics of a specific deuteromycete (fungi imperfecti).

SUGGESTION After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 3

SAPROPHYTIC FUNGI

Section I. INTRODUCTION

3-1. REASONS FOR STUDYING

Saprophytic fungi are studied for two major reasons. First, because they often have an appearance similar to known fungal pathogens. Second, because under special conditions they may cause overt disease (opportunistic infections).

3-2. POTENTIAL CLINICAL IMPORTANCE

Even though saprophytic fungi isolated from clinical specimens may be "just contaminants," they should NOT be routinely labeled as such without first considering the following: (1) presence of a large number of colonies of a specific fungus or repeated isolation of the same fungus from consecutive specimens; (2) presence of the vegetative form of the fungus in the direct examination; and (3) patient's clinical history and current state of health. The laboratory determines points (1) and (2), whereas point (3) requires coordination with the physician. The infectious disease specialist should be consulted to assist with the evaluation.

3-3. OPPORTUNISTIC INFECTIONS

Opportunistic infections are becoming more of a problem because of increasing use of instrumentation (catheters, inhalation therapy machines, and so forth), antibiotics, and other drugs that bypass or reduce the natural resistance of the host. Because of these conditions, organisms that were once considered as contaminants are now recognized as being capable of causing severe disease.

Section II. ZYGOMYCETES

3-4. INTRODUCTION

The zygomycetes are characterized by rarely septate hyphae, sporangiospores, and sexual zygospores. The zygomycetes include some genera which are ordinarily harmless, but can cause serious diseases in diabetics and other people with lowered resistance. These diseases are collectively called mucormycoses because the causative fungi all belong to the order Mucorales. Mucormycoses are serious, often fatal, diseases of the respiratory tract, blood vessels, brain, or other organs. Some of the organisms that may cause mucormycoses include Mucor, Rhizopus, Absidia, and Syncephalastrum species.

3-5. MUCOR SPECIES

The Mucor colony is rapid-growing, forming a cottony surface that fills the culture plate in five to seven days. Aerial mycelium is white, later becoming gray to brown. The texture is extremely fluffy. Microscopically, branching sporangiophores arise from rarely septate hyphae. Spherical sporangiospores are seen within the sporangium and around the columella (a dome-shaped structure located at the top end of the sporangiophore). Rhizoids (specialized hyphae that resemble roots and whose presence or absence and location are key identification characteristics) are not produced by Mucor spp. (figure 3-1).

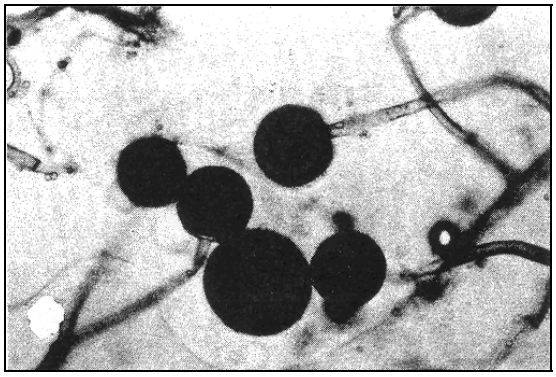


Figure 3-1. Microscopic morphology of Mucor species.

3-6. RHIZOPUS SPECIES

The Rhizopus colony is rapid growing, with voluminous white to gray aerial mycelium presenting a peppered appearance. The texture is extremely fluffy. Microscopically, sporangiophores are long, unbranched, and clustered at nodes opposite rhizoids that form along a horizontal runner (stolon). The key identification characteristic for Rhizopus is the location of these rhizoids. Rhizopus has rhizoids that are located opposite of the sporangiophores. In other zygomycetes, rhizoids are not present or are in different location. Sporangia are dark-walled, spherical, and filled with round hyaline spores. A columella, which is thought to supply nutrients to the organism, is present. The columella is often obscured by sporangiospores (figure 3-2).

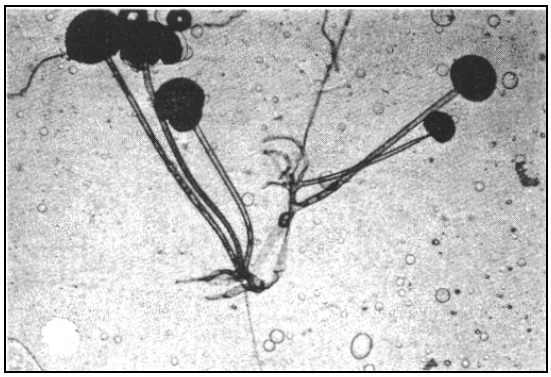


Figure 3-2. Microscopic morphology of Rhizopus species.

3-7. SYNCEPHALASTRUM SPECIES

Syncephalastrum colonies (figure 3-3) are rapid growing. They are white at first but turn gray with age. The texture is dense and fluffy to cottony in appearance. The large, rarely septate hyphae are highly branched. Short, branched sporangiophores are erect and terminate in globose to oval vesicles giving rise to fingerlike tubular sporangia called merosporangia, each of which has a single row of 2 to 10 sporangiospores.

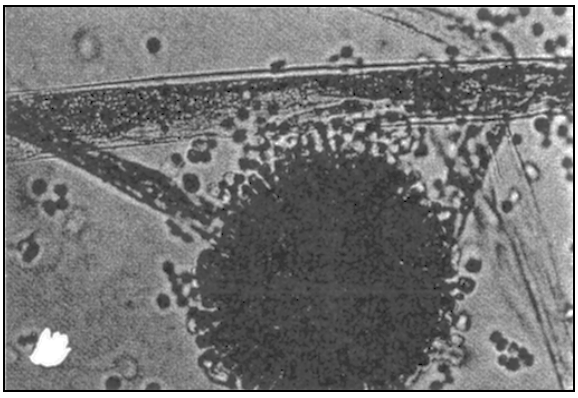


Figure 3-3. Microscopic morphology of Syncephalastrum species.

3-8. ABSIDIA SPECIES

The Absidia colony is gray with a coarse, woolly texture. Microscopically, rhizoids are present, but the sporangiophores arise between the nodes of the stolon instead of opposite the nodes as in Rhizopus. Sporangia are pear-shaped, filled with round to oval spores, and contain a columella (figure 3-4).

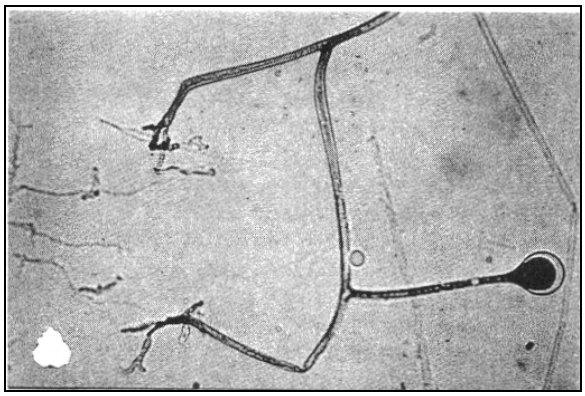


Figure 3-4. Microscopic morphology of Absidia species.

Section III. DEUTEROMYCETES

3-9. INTRODUCTION TO DEUTEROMYCETES (FUNGI IMPERFECTI)

Fungi that have no known sexual cycle of reproduction are classed as deuteromycetes. Since the sexual cycle is either nonexistent or undiscovered in these organisms, they are commonly called the "imperfect fungi." The only characteristic shared by all the fungi of this group is the absence of a known sexual cycle. Their appearance may resemble organisms in any of the other three classes. Most of the deuteromycetes, however, look like ascomycetes, with septate hyphae and similar asexual spores. Members of these imperfect fungi are continually being placed in other groups as their elusive sexual cycles are discovered. Many important pathogens, however, are still considered fungi imperfecti. Included in this group of fungi are Alternaria, Cladosporium, Drechslera, Curvularia, Penicillium, Paeclomyces, Aspergillus, Nigrospora, Scopulariopsis, Trichoderma, Sepedonium, and Fusarium species.

3-10. ASPERGILLOSIS

a. Aspergillosis is a complex group of diseases caused by certain species within the genus Aspergillus. Sites of infection include the lung, ear, sinus, eye, and skin. Pulmonary aspergillosis, the most common form of disease, is usually caused by Aspergillus fumigatus. Three forms of this disease are recognized.

(1) Allergic aspergillosis, which may cause asthma-like symptoms or bronchitis, with plugging of the bronchi by mucus.

(2) Aspergilloma, or "fungus ball," caused by growth of the fungus in preexisting lung cavities.

(3) Invasive aspergillosis, in which the fungus invades the tissue. The latter form of the disease is usually found in debilitated patients when the immune system has been impaired.

b. Clinical symptoms of pulmonary aspergillosis include fever, and cough with blood-tinged sputum. Infection occurs from inhaling spores of the fungus or by inoculation with material containing the fungal elements.

c. Aspergillus is found in soil, air, plants, animals, and has a worldwide distribution. Because it is common in the environment and acts as an opportunistic pathogen, aseptic procedures should be exercised whenever possible during collection and transport of clinical specimens. When attempting to isolate organisms from sputum, multiple specimens must be collected, preferably early in the morning and on consecutive days. Direct microscopic examination of clinical specimens reveals long, branching, hyaline, septate hyphae. Specimens are inoculated to SDA and incubated at 25º C to 30º C. Colonies appear in three to four days. Aspergillus is inhibited by media containing cycloheximide. Mycosel™ medium, which contains this antimicrobial agent, should not be used. The diagnosis of aspergillosis is usually made by clinical symptoms, demonstration of hyphae in clinical specimens, and repeated isolation of the same species from the same anatomic site.

d. Macroscopic examination the surface color of the colony is white, becoming gray-green with age. The surface texture is cottony.

e. Microscopic examination. Conidiophores are thin-walled, smooth, green, and end in a hemispherical vesicle. The vesicle is a swollen part of the cell located at the end. Phialides, the cells from which the conidia originate, are flask-shaped. Conidia are single-celled, globose, echinulate (covered with spines), thin-walled, and are 2 to 3.5 mcm in diameter (figure 3-5).

f. Unlike other species of the genus, Aspergillus fumigatus is thermotolerant and grows very well at 45º C or higher. Thermotolerance and specimen site information may be used as a screening procedure for this organism.

Figure 3-5. Microscopic morphology of Aspergillus species.

3-11. ALTERNARIA

a. Alternaria colonies are rapid growing, cottony, and gray to black. The conidiophores are dematiaceous. Dematiaceous refers to the brown or black color imparted to conidia or spores due to the presence of melanin. The conidia of Alternaria spp. develop branching chains at the apex of the conidiophore, with the youngest conidium at the apex of each chain. The conidia are dematiaceous and muriform (possessing both vertical and horizontal septations) (figure 3-6).

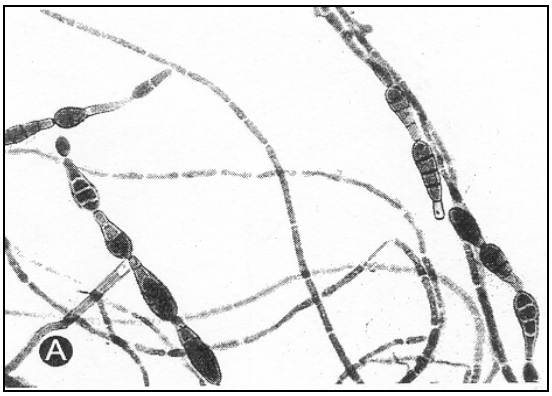


Figure 3-6. Microscopic morphology of Alternaria species.

b. Isolates of Alternaria are occasionally encountered in the clinical laboratory. Several species have been reported as pathogens of man. However, in most instances, Alternaria spp. are recovered as skin contaminants and do not contribute to a disease process.

3-12. CLADOSPORIUM

a. Cladosporium isolates are rapid growing, velvety or cottony, and are usually olive-gray to olive-brown or black. Tall, erect dematiaceous conidiophores arise from the mycelium. At the apex of the branching, conidiophores form branching chains consisting of one to several celled, smooth or rough, dematiaceous blastoconidia. The conidium at the bottom of the chains is larger, and due to its appearance, is commonly referred to as a shield cell (figure 3-7).

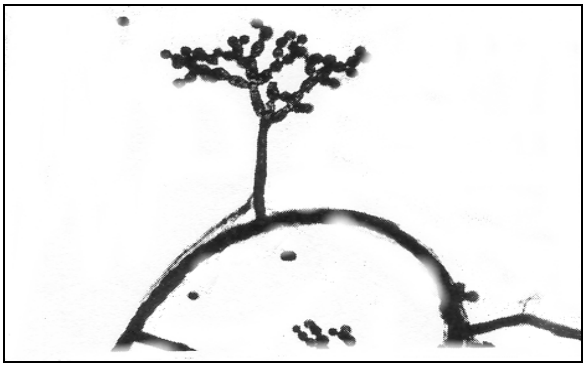


Figure 3-7. Microscopic morphology of Cladosporium species.

b. Cladosporium is commonly recovered in the clinical laboratory. Most of the more common species of Cladosporium are soil fungi. The pathogenic species can be differentiated from nonpathogens by tests for digestion of Loeffler's coagulated serum medium or for liquefaction of gelatin. These tests are usually negative for pathogens.

3-13. DRECHSLERA

a. Drechslera species form rapid-growing, woolly, gray-to-black colonies. The conidiophores are dematiaceous, solitary or in groups, simple or branched, septate, and geniculate. The term geniculate refers to being bent like a knee. The dematiaceous, oblong-to-cylindrical conidia are multicelled (figure 3-8).

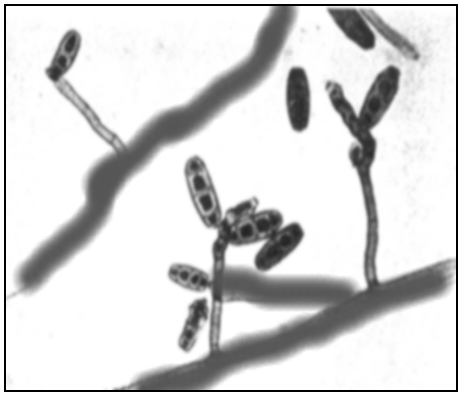


Figure 3-8. Microscopic morphology of Drechslera species.

b. Drechslera is frequently recovered in the clinical laboratory. Members of this genus are common soil organisms and some species are plant pathogens. Several Drechslera species cause opportunistic infections in humans, including meningitis, cutaneous, eye, nasal, and pulmonary infections.

3-14. CURVULARIA

a. Curvularia colonies are rapid growing, woolly, and gray to grayish black or brown. The conidiophores of Curvularia are brown, simple or branched, solitary or in groups, and borne laterally or terminally from the septate hyphae. The poroconidia are usually curved, with one of the central cells being larger and darker than the other cells (figure 3-9).

b. Curvularia spp. has been implicated in a number of opportunistic infections. Members of this genus cause endocarditis, eye infections, mycetoma, and pulmonary disease.

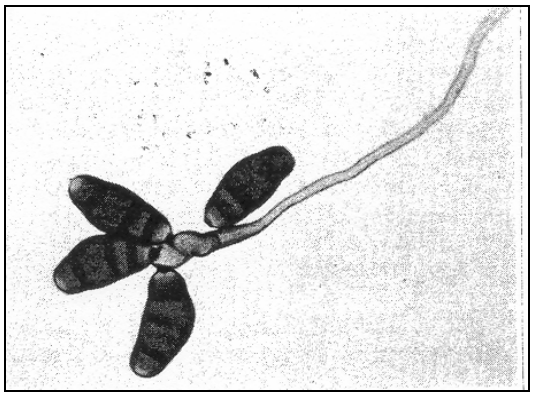


Figure 3-9. Microscopic morphology of Curvularia species.

3-15. PENICILLIUM

a. Penicillium colonies are rapid growing. White colonies change with maturation to colors characteristic of each species. The most commonly isolated species are blue-green or yellow-green. The conidiophores are erect, distinct, usually branched, smooth to rough, and hyaline to colored. The branches, called metulae, may be either symmetrical or asymmetrical in relation to the conidiophore. Flask-shaped, hyaline phialides are borne at the apex of the terminal metulae. Each phialide produces chains of one-celled phialoconidia that may be hyaline to dark and smooth to rough-walled (figure 3-10).

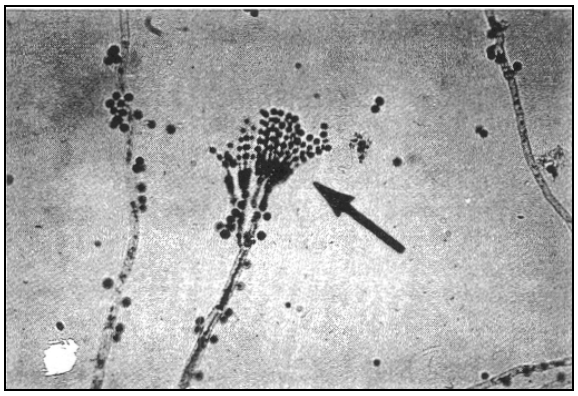


Figure 3-10. Microscopic morphology of Penicillium species.

b. Isolates of Penicillium are commonly isolated in the clinical laboratory and are considered among the most prolific and ubiquitous groups of fungi. In most instances, they are contaminants, not pathogens. Infections have been reportedly associated with different stages of debilitation and with specimens from numerous anatomical sites. Repeated isolation and demonstration of fungal elements in tissue are mandatory before a strain of Penicillium species can be considered an opportunistic pathogen.

3-16. PAECILOMYCES

a. Paecilomyces species resemble Penicillium species in growth characteristics and in some elements of microscopic appearance. The primary microscopic characteristic of Paecilomyces species is a conidiophore with graceful, elongated, beak-like phialides curving outward, away from each other. Chains of phialoconidia generally are oval and may become enlarged in order chains (figure 3-11).

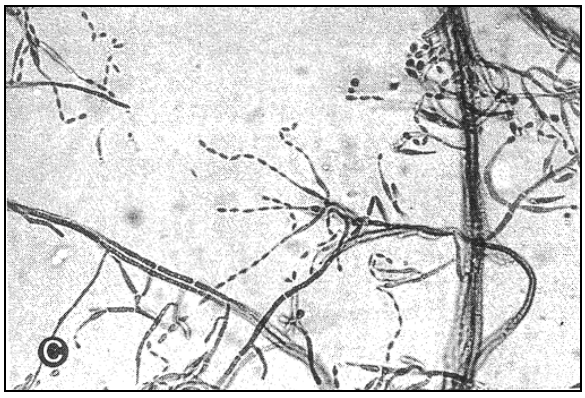


Figure 3-11. Microscopic morphology of Paecilomyces species.

b. Reports of patients with paecilomycosis usually give a history of exposure to infectious foreign material or traumatization of body tissue that later becomes infected.

3-17. NIGROSPORA

Nigrospora colonies are rapid-growing, woolly to cottony, and white to gray in color. The hyphae are septate. Conidiophores are simple, unbranched and short, with inflated end or vesicle. The conidium is black, spherical, and borne on the end of the vesicle (figure 3-12).

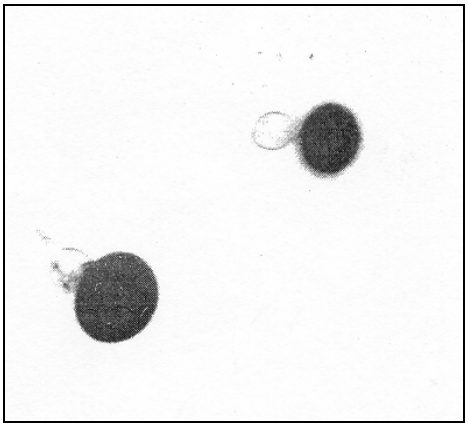


Figure 3-12. Microscopic morphology of Nigrospora species.

3-18. SCOPULARIOPSIS

a. Scopulariopsis colonies are rapid-growing, initially white in color, turning tan to brown with age. Texture is flat and powdery. Members of the species produce globose to pyriform annelloconidia in chains from annellides that are either solitary or grouped on a conidiophore. Mature conidia are often rough and echinulated (spiny) (figure 3-13).

Figure 3-13. Microscopic morphology of Scopulariopsis species.

b. Scopulariopsis spp. are commonly isolated in the clinical laboratory. They are often contaminants in humans but rarely pathogenic.

3-19. TRICHODERMA

Trichoderma colonies are rapid growing and white to green in color. Texture is cottony. Hyphae are septate and branched. Conidiophores are short and branched with flask-shaped phialides. Phialoconidia are seen as clusters of single-celled microconidia (figure 3-14).

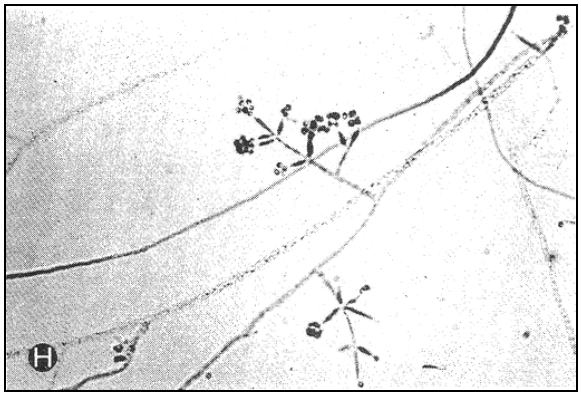


Figure. 3-14. Microscopic morphology of Trichoderma species.

3-20. FUSARIUM

a. Fusarium species display colonies that are fluffy in texture and of variable color (typically violet to purple). Microscopically, Fusarium spp. are characterized by the production of canoe-shaped, multicelled macroconidia and one or two-celled hyaline microconidia, usually held together in mucus balls. The macroconidia generally are borne in banana-like clusters, which dislodge easily and float free from the hyphae. Not all strains demonstrate both types of conidial production (figure 3-l5).

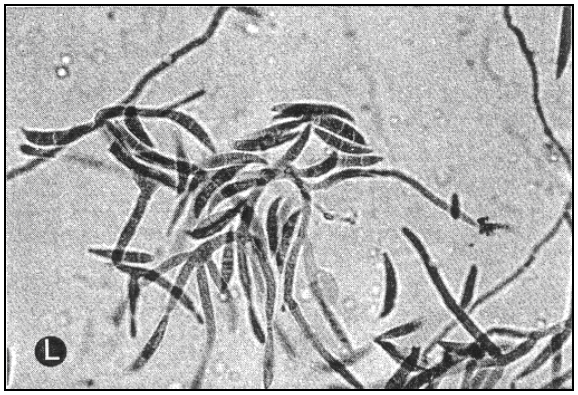


Figure 3-15. Microscopic morphology of Fusarium species.

b. Fusarium is commonly cultured from specimens in the clinical laboratory. Best known as a laboratory contaminant, it is now recognized as an opportunistic pathogen causing eye infections in burn patients, recipients of renal transplants, postsurgical patients, and in animals (primarily dogs).

3-21. SEPEDONIUM

Sepedonium colonies are rapid growing, white in color and have a cottony texture. The species produces spiny, tuberculate conidia similar to the conidia of the pathogen Histoplasma capsulatum. Sepedonium do not convert to a yeast at 35º C to 37º C. Some species have amber-colored conidia. The asexual form of the organism may also be present in the same culture with sepedonium (figure 3-16).

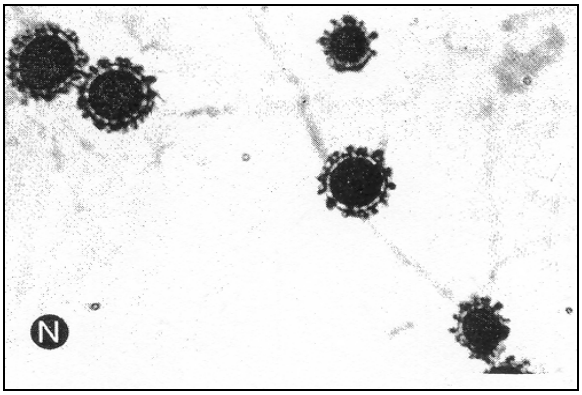


Figure 3-16. Microscopic morphology of Sepedonium species.

Continue with Exercises

EXERCISES, LESSON 3

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Opportunistic infections caused by saprophytic fungi are:

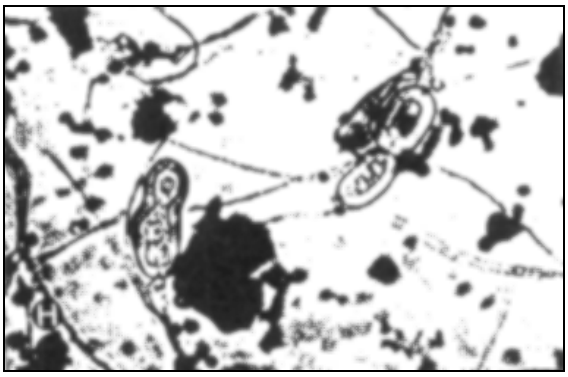
   a. Of little concern to the laboratory specialist.

   b. Rarely capable of causing disease.

   c. Of increasing importance due to increased instrumentation.

   d. Of increasing importance because new strains are being identified.

2. Culture of a specimen from a patient with pulmonary symptoms produces a colony whose microscopic morphology resembles the illustration below. The fungus most probably is:



   a. Rhizopus.

   b. Aspergillus.

   c. Mucor.

   d. Candida.

3. The group of fungi responsible for mucormycoses is:

   a. Zygomycetes.

   b. Ascomycetes.

   c. Basidiomycetes.

   d. Deuteromycetes.

4. Deuteromycetes are commonly called "fungi imperfecti" because they:

   a. Are dimorphic.

   b. Have no known sexual cycle.

   c. Are not true fungi.

   d. Cladosporium spp.

5. Shield cells are characteristic of which of the following?

   a. Alternaria spp.

   b. Paecilomyces spp.

   c. Dreschlera spp.

   d. Cladosporium spp.

6. An organism commonly appearing culture as a contaminant is:

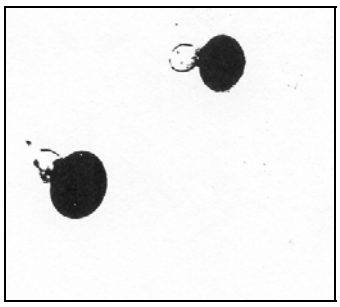
   a. Alternaria.

   b. Aspergillus.

   c. Penicillium.

   d. Absidia.

7. The organism illustrated below is:



   a. Paecilomyces.

   b. Nigrospora.

   c. Cladosporium.

   d. Penicillium.

8. When reviewed microscopically, Fusarium is characterized by:

   a. Canoe-shaped macroconidia.

   b. Truncate annelloconidia.

   c. Elongated phialides.

   d. Whorled conidia.

9. An organism producing spiny, tuberculate conidia similar to the conidia of the pathogen Histoplasma capsulatum is:

   a. Nigrospora spp.

   b. Sepedonium spp.

   c. Fusarium spp.

   d. Penicillium spp.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 3

1. c (para 3-3)
2. b (figure 3-5)
3. a (para 3-4)
4. b (para 3-9)
5. d (para 3-12a)
6. c (para 3-15b)
7. b (figure 3-12)
8. a (para 3-20a)
9. b(para 3-21)

End of Lesson 3

LESSON ASSIGNMENT

LESSON 4	Yeasts of Medical Importance.
LESSON ASSIGNMENT	Paragraphs 4-1 through 4-10.
LESSON OBJECTIVES	Upon completion of this lesson, you should be able to

4-1. Select the statement that correctly describes a characteristic of Candid*a* organisms.

4-2. Select the statement that correctly describes a characteristic of Cryptococcus neoformans.

4-3. Select the statement that correctly describes a characteristic of Geotrichum candidum.

4-4. Select the statement that correctly describes a characteristic of genus Torulopsis.

4-5. Select the statement that correctly describes a characteristic of Saccharomyces cerevisiae.

SUGGESTION After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 4

YEASTS OF MEDICAL IMPORTANCE

4-1. INTRODUCTION

a. Yeasts are unicellular budding fungi that reproduce by sexual or asexual means. The clinically significant genera of yeasts for man include Candida, Cryptococcus, Saccharomyces, Torulopsis, and Trichosporon. Species of the genus Geotrichum , which are true molds, were once classified under the genus Trichosporon and are still identified with procedures commonly used for yeasts.

b. The incidence of infections caused by yeasts has markedly increased due to therapeutic measures reducing the natural immunity of the host. Burn victims, cancer patients, and individuals receiving antibiotics are representatives of this high-risk group. Yeasts often colonize humans as normal flora but can cause complications and disease. In the past, it was necessary to identify only a few species of yeasts, but repeated isolation of a specific yeast, combined with clinical information, can influence the physician in patient management decisions. Characteristics of yeasts commonly isolated in the clinical laboratory are discussed in the following paragraphs.

4-2. CANDIDIASIS

a. Candidiasis is a disease process usually caused by Candida albicans. In a compromised host, any species within the genus can be pathogenic given the right conditions. Candida spp. often invades moist areas of the skin including the groin, scrotum, fold of the buttocks, under the breast, in the umbilicus, and in interdigital spaces. Characteristic lesions have a red "scalded skin" appearance and a scalloped border. Satellite pustular lesions surround the primary lesion. Dry, scaly lesions may also occur. On direct examination, budding blastoconidia and pseudohyphae are seen. If left untreated, Candida spp. spread easily *to* other areas of the body. Underlying disease (diabetes, chronic alcoholism, and so forth) or environmental factors that lead to increased moisture (tight clothing in hot climates, continual wear of a wet bathing suit, continual immersion of hands in water) contribute *to* the disease process.

b. Candida diaper rash is initiated by the colonization of Candida albicans in the diaper-covered area of infants. An allergic reaction precedes invasion of the epidermis. Spreading to other areas of the body is quite common, especially the eyes and mouth. Candida diaper rash is common when poor hygiene is practiced. Constant exposure to unclean, wet diapers should be avoided. When diaper rash occurs, care should be taken to keep the infected area dry.

c. Mucocutaneous candidiasis is almost entirely caused by Candida albicans. However, other species of Candida may be involved in seriously compromised patients. The disease thrush is caused by colonization of Candida in the mouth. It is characterized by white to gray membranous patches, resembling cottage cheese, which involve the tongue and mucomembranes of the oropharynx. It is often found in newborn infants who are infected by strains of Candida albicans from the mother's vagina. This causes a primary oral infection in the newborn before competing flora are established. In older children and adults, poor nutrition, diabetes, and other underlying disease may influence the disease process. Perleche, a Candida infection of the corners of the mouth, is closely associated with thrush.

d. Vaginal candidiasis is quite common. It is characterized by a yellow, milky, or cheesy discharge. The mucous membrane of the vagina may have a mild redness or inflammation with pustules, ulcers, and severe itching. The incidence of Candida vaginitis increases with pregnancy, in diabetes, and prolonged antibiotic therapy. Environmental factors such as wearing a wet swimming suit for a prolonged length of time, tight-fitting pants, and nylon type underwear that does not allow adequate air circulation can also increase the incidence of vaginal candidiasis.

4-3. CANDIDA ALBICANS

Colonies grow aerobically at 25º C to 30º C with some growth visible as early as 24 to 36 hours. Colonies are usually stark white, but may become cream colored or tan with age. They are glabrous, creamy, or membranous, and may have a fringe of submerged hyphae. Microscopic examination shows the presence of globose to ovoid blastoconidia and well developed pseudohyphae. Production of germ tubes and spherical chlamydospores on starvation media is a useful diagnostic characteristic (figure 4-1).

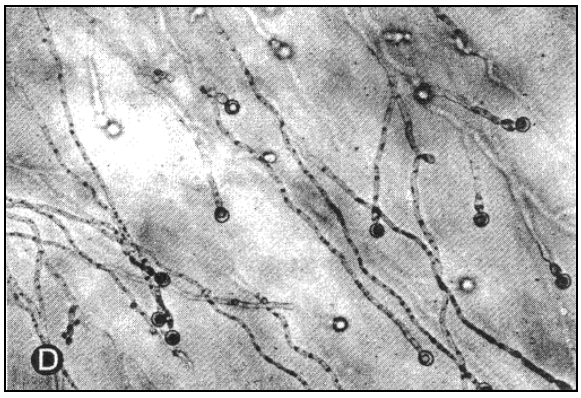


Figure 4-1. Microscopic morphology of Candida albicans.

4-4. CANDIDA TROPICALIS

Colonies of Candida tropicalis may be observed within 24 to 72 hours after inoculation. The colonies are white with an occasional blue-green center. The texture is creamy with a marked feathery periphery. Microscopic morphology includes chaining, ovoid blastoconidia alongside long, branching pseudohyphae. Although not commonly seen, "tear drop" shaped chlamydospores may appear upon prolonged incubation.

4-5. DIFFERENTIATION OF CANDIDA SPECIES

a. Tests, to determine the ability of a yeast species to use a carbohydrate as the sole source of carbon in a chemically defined medium, have long been a mainstay of yeast taxonomists and have become an essential step in yeast identification in the clinical mycology laboratory. Most commercial products now available for identifying yeasts rely heavily on carbohydrate assimilation tests. Carbohydrate fermentation tests are also useful tests for yeast identification. However, these tests are more variable and less dependable than carbohydrate assimilation tests. The only reliable evidence for carbohydrate fermentation is the production of gas.

b. Characteristic microscopic morphologies of other Candida species that may be isolated in the clinical laboratory include:

(1) Candida guilliermondii. Rudimentary or well-developed pseudohyphae may be seen. When well developed, the pseudohyphae are slender, often curved and appear as ovoid, or chains of elongated blastoconidia.

(2) Candida krusei. Pseudohyphae consisting of long cells with tree-like branching are often seen with chains of blastoconidia arising at points of branching. Curved pseudohyphae, with scarce blastoconidia, are seen in some strains.

(3) Candida parapsilosis. Thin pseudohyphae, usually branched, bearing spirals of a few blastoconidia. The pseudohyphae appear as curved and giant cells.

(4) Candida paratropicalis. Resembles Candida tropicalis.

(5) Candida pseudotropicalis. Pseudohyphae typically abundant and branched. Chains of blastoconidia are also present.

(6) Candida stellatoidea. Resembles Candida albicans. Both Candida albicans and Candida stellatoidea produce germ tubes, but Candida albicans can assimilate sucrose whereas Candida stellatoidea does not.

4-6. GEOTRICHUM CANDIDUM

a. Geotrichum candidum is most frequently isolated from clinical specimens of the upper respiratory tract. It is found in oral infections similar to thrush, in purulent lesions associated with bronchial infections and in pulmonary lesions with upper respiratory tract congestion.

b. Colonies appear within 24 to 72 hours after inoculation. Geotrichum is a filamentous fungus whose initial growth may be glabrous, and creamy to pasty, but becomes velvety or fuzzy with aging and repeated subculture. Geotrichum produces true hyphae and arthroconidia but neither pseudohyphae nor blastoconidia, thus differing morphologically from the yeast genus, Trichosporon. Arthroconidia are seen in rectangular, consecutive chains. Germ tubes may form on a corner of germinating arthroconidia (figure 4-2).

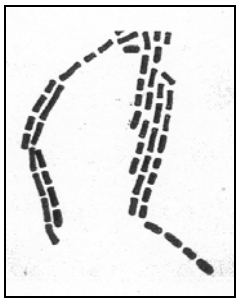


Figure 4-2. Microscopic morphology of Geotrichum candidum.

4-7. CRYPTOCOCCUS

a. Of the eight recognized species of Cryptococcus, the only true pathogen is Cryptococcus neoformans. Infections caused by C. neoformans are exogenous; the yeast lives naturally in soil contaminated with bird droppings, notably from pigeons and other seed-eating birds. Meningitis is the most frequently recognized type of cryptococcal infection, followed in frequency by localized abscesses or granulomas in lungs, brain, lymph nodes, skin, or bones (figure 4-3). Diffuse pulmonary infection is perhaps the most common type of cryptococcal infection, although it is often asymptomatic and unrecognized. The respiratory tract is believed to be the portal of entry for most, if not all cryptococca1 infections.

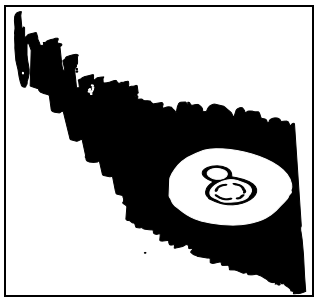


Figure 4-3. Encapsulated cells of Cryptococcus neoformans in India ink.

b. Cryptococcus colonies are often mucoid, becoming dull and drier with age. Colonies appear within 24 to 72 hours and are pale buff, becoming tan or brown with age. The genus does not ordinarily form mycelium. Globose dark-walled blastoconidia are seen.

c. C. neoformans can be differentiated from other Cryptococcus spp. by:

(1) Its ability to grow at 37º C (although four other species also grow at 37º C and two are variable).

(2) The production of brown colonies on birdseed agar.

(3) A characteristic assimilation pattern.

(4) Its pathogenicity for experimental animals.

d. Serologic latex agglutination tests may be useful in screening for cryptococcus antigen in CSF, serum, and urine specimens.

4-8. RHODOTORULA

a. Rhodotorula colonies resemble cryptococcus colonies in rate of growth and colony topography. Their cell sizes and shapes are also similar. Its nonpathogenicity, serotype, and conspicuous carotenoid pigment distinguish Rhodotorula as a distinct genus. Rhodotorula is a normal colonizer of moist skin and may be an opportunistic pathogen.

b. Growth occurs within 24 to 72 hours as orange to red colonies with a butyrous texture. Microscopic morphology shows globose to ovoid blastoconidia with no pseudohyphae or hyphae.

4-9. TORULOPSIS

a. Torulopsosis is a disease process caused by the opportunistic organism Torulopsis glabrata. It is part of the normal flora of the mouth, nose, gastrointestinal tract and vagina. Recovery from these sites in an uncompromised host is routinely ignored. Torulopsis glabrata causes disease mostly in hospitalized patients who already have an existing serious disease. The factors that increase colonization and facilitate tissue invasion are similar to those for Candida albicans. The usual portals of entry for the compromised patient are intravenous or intraurethral catheters.

b. Patients with torulopsosis experience a sudden onset of fever that may be accompanied by hypotension. Although serious complications may arise, infections are rarely fatal. The etiological agent is rarely recovered from tissue. The prognosis depends upon ability to identify and eliminate the portal of entry, and the presence or absence of underlying disease. Sepsis caused by Torulopsis glabrata is usually seen in patients receiving an increased amount of nutrients by intravenous feeding (hyperalimentation). Prompt removal of the contaminated catheter usually cures the sepsis without any further treatment. A new catheter can be inserted at a different site without detriment to the patient. Torulopsis glabrata infection of the urinary tract is commonly found in patients with diabetes mellitus, urinary tract obstruction, and following the use of instrumentation. Torulopsis glabrata can cause shallow ulceration in the esophagus or gastrointestinal tract; especially in patients with a hematological malignancy. It is unclear if infection at these sites leads to dissemination.

c. Torulopsis is a common isolate from urine and other specimens. It is considered a symbiont of humans, but has been documented as causing pyelo/nephritis, pneumonia, septicemia, and meningitis in immunocompromised patients.

d. Growth occurs within 24 to 48 hours as white to yellow colonies with a butyrous texture. Microscopic morphology includes small, ovoid blasto/conidia, with no hyphae, pseudohyphae, or capsule observed.

4-10. SACCHAROMYCES

a. Saccharomyces cerevisiae is responsible for occasional cases of thrush, vulvovaginitis and urinary tract infections. It is an ascosporogenous yeast. The ascospores may be demonstrated by use of Henrici's ascospore stain to differentiate them from vacuoles.

b. Colonial growth is evident in 24 to 72 hours. Colonies resemble Candida in color and texture. Microscopically, the cells appear oval to spherical, and may exist as either haploids or diploids. Cells may form short chains and elongate as rudimentary pseudohyphae. Ascospores, one to four in number, are in either tetrahedral or linear arrangement, and are gram-negative. Vegetative cells, on the other hand, will stain gram-positive.

Continue with Exercises

EXERCISES, LESSON 4

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Which of the following would be considered at high risk of yeast infections?

   a. Newborns.

   b. Burn victims.

   c. Lab personnel.

   d. Hospital patients.

2. A diagnostic characteristic of Candida albicans is the production of:

   a. Chlamydospores on starvation media.

   b. Cream colored colonies.

   c. Pseudohyphae.

   d. Chains of blastoconidia.

3. Procedures used to differentiate Candida species include:

   a. Microscopic morphology.

   b. Carbohydrate assimilation tests.

   c. Carbohydrate fermentation tests.

   d. All of the above.

4. An organism with microscopic morphology resembling Candida albicans is:

   a. Candida krusei.

   b. Candida stellatoidea.

   c. Blastoconidium.

   d. Candida parapsilosis.

5. What are the characteristics asexual cell produced by Geotrichum candidum?

   a. Chlamydospore.

   b. Pseudohyphae.

   c. Blastoconidium.

   d. Arthroconidium.

6. Infections caused by Cryptococcs neoformans are most often located in the

   _____ and the _____.

7. Differentiation of Cryptococcus neoformans from nonpathogenic members of the species can readily be done by observation of colonial morphology.

   a. True.

   b. False.

8. Which of the following organisms is identified by use of Henrici's ascospore stain?

   a. Saccharomyces cerevisiae.

   b. Cryptococcus neoformans.

   c. Geotrichum candidum.

   d. Candida albicans.

9. Which of the following is characteristic of Rhodotorula?

    a. Considered a pathogen.

    b. Carotenoid colonies.

    c. Submerged hyphae.

    d. Growth in body fluids.

10. An immunocompromised person may display pyelonephritis caused by:

    a. Trichosporon.

    b. Any nonpathogen.

    c. Torulopsis.

    d. Cryptococcus.

11. Candida infections are most likely to occur in _____ areas of the skin.

12. Candida albicans is the causative agent for:

    a. Thrush.

    b. Diaper rash.

    c. Vaginitis.

    d. All of the above.

13. The symptoms of infections with Torulopsis glabrata include:

    a. Headache.

    b. Malaise.

    c. Hypertension.

    d. Fever.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 4

1. b (para 4-1b)
2. a (para 4-3)
3. d (paras 4-5a, b)
4. b (para 4-5b)
5. d (para 4-6b)
6. Meninges, lungs. (para 4-7a)
7. b (para 4-7c)
8. a (para 4-10a)
9. b (para 4-8a)
10. c (para 4-9c)
11. moist. (para 4-2a)
12. d (paras 4-2b, c, d)
13. d (para 4-9b)

End of Lesson 4

LESSON ASSIGNMENT

LESSON 5	Cutaneous Fungi.
LESSON ASSIGNMENT	Paragraph 5-1 through 5-10.
LESSON OBJECTIVES	Upon completion of this lesson, you should be able to:

5-1. Select the statement that correctly describes a morphologic characteristic of a specific superficial fungus.

5-2. Select the statement that correctly describes a characteristic of a specific dermatophytic fungus.

5-3. Select the statement that correctly describes the clinical aspects of the disease.

5-4. Select the fungus responsible for a specific dermatophytic disease.

SUGGESTION: After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

CUTANEOUS FUNGI

Section I. SUPERFICIAL FUNGI

5-1. INTRODUCTION

Superficial fungi do not cause life-threatening infections in patients, but because the body area affected is the outer layer of the skin, the psychological problems of affected individuals usually require attention. Alleviation of psychological discomfort normally includes treatment of the infection.

5-2. MALASSEZIA FURFUR

a. Malassezia furfur is found worldwide but is most prevalent in tropical areas. It causes tinea versicolor, a disease affecting smooth body skin areas. Tinea versicolor is a chronic infection usually affecting young adults. It is most often asymptomatic and is seen as scaly areas on the upper body. Early lesions are pink and appear irritated. Older lesions are hypopigmented (showing diminished pigmentation), particularly after exposure to the sun. When placed under a Wood's lamp (fluorescent light) in a "dark room" most infected areas fluoresce dull-red to orange.

b. Laboratory identification of Malassezia furfur is usually made by direct examination of skin scrapings from the infected site. Microscopic examination shows clusters of small, thick-walled, round blastoconidia with mycelial fragments. The combination of round blastoconidia and mycelia gives an appearance called "spaghetti and meatball" effect. Cultures are usually unsuccessful and not required to establish a diagnosis of tinea versicolor, but sometimes isolation may be necessary or desirable. Since Malassezia furfur is a lipophilic organism, it requires the presence of a fatty material overlay for growth and inoculated media is commonly overlaid with sterile olive oil to enhance growth. When growth occurs, colonies are cream-colored, glossy, and raised, but later become dull, dry, and beige-colored. Microscopic morphology shows globose to ellipsoidal hyaline cells (3 to 7 mcm). Mycelia are not usually formed in culture, but some cells may produce germ tubes (figure 5-1).

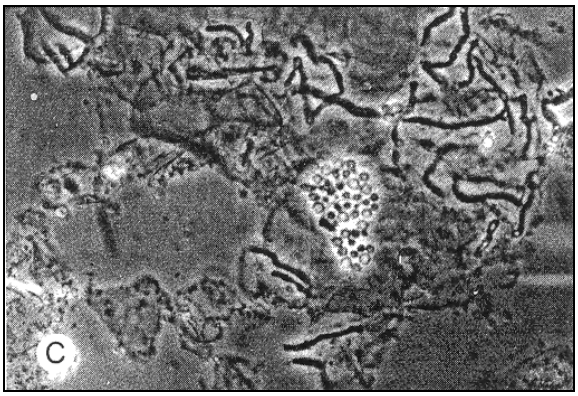


Figure 5-1. Microscopic morphology of Malassezia furfur.

5-3. EXOPHIALA WERNECKII

a. Exophiala werneckii (Cladosporium werneckii) is found worldwide but most commonly in the tropics and subtropics. It is the cause of tinea nigra, an infection that presents as black blotches on the palms of hands, rarely on other body areas. The disease is largely asymptomatic but can be confused with malignant melanoma.

b. Direct examination of skin scrapings from the suspected infection on site demonstrates light brown to dark-green branching septate hyphae and possibly chlamydospores. Colonial growth on isolation media is black and shiny, with a yeast-like surface and a black reverse. Microscopic morphology of colonies shows olive-colored, budding blastoconidia, and thick-walled septate hyphae. There are short conidiophores with one- or two-celled annelloconidia. Older cultures may show branching chains of annelloconidia.

5-4. PIEDRA HORTAE

a. Piedra hortae is distributed worldwide but is most prevalent in tropical regions. It causes black piedra characterized by hard, brown, or black nodules on shafts of scalp hair. Although the patient feels no discomfort, the hair feels "gritty" and a metallic sound may be heard when combed.

b. Infected scalp hairs are examined directly for the presence of nodules that consist of tightly packed masses of wide, dark brown, septate, and branching hyphae. Crushed nodules release two to eight single celled ascospores. Culture is not required for diagnosis. Colonies are dark brown to black, with a glabrous or smooth surface and an unremarkable reverse. Microscopic morphology includes thick-walled, brownish hyphae with chlamydospores. Asci and ascospores are not usually produced on routine media.

5-5. TRICHOSPORON BEIGELLI

a. Trichosporon beigelli is distributed worldwide but is most prevalent in the tropics. It causes white piedra. This disease is characterized by the presence of hard white nodules on the hair shaft. The hairs on the scalp are generally affected, but occasionally the beard of upper lip may be involved. The nodes are thought to arise near the scalp. However, due to normal hair growth, they are commonly observed on the distal portion of the hair. The nodes may be just visible to the naked eye or felt as pinhead size gritty masses adhering to one side, or surrounding the hair shaft. More than one nodule may be present on each infected hair. The intervening portions of the hair remain normal and the skin is not involved. There is no fluorescence under ultraviolet light.

b. Infected scalp hairs are examined directly for the presence of an ectothrix infection (defined below) with hyphae, arthroconidia, and blastoconidia. The fungus grows rapidly and in a few days produces a cream-colored yeast-like colony that becomes yellowish-gray, dry and wrinkled at two to four weeks of age. Microscopic morphology shows branching hyphae with arthroconidia. Ovoid to ellipsoidal blastoconidia and pseudohyphae are also produced. The organism is commonly confused with the mold Geotrichum due to a similar colonial morphology and the presence of hyphae with arthroconidia. Trichosporon, however, additionally produces blastoconidia and pseudohyphae, making this organism a true yeast.

c. There are two basic types of hair infection: ectothrix and endothrix. An ectothrix infection is characterized by arthroconidia on the outside of the hair and mycelium within the hair shaft. It therefore has fungal elements on both the outside and inside of the hair shaft. An endothrix infection is characterized by arthroconidia within the hair shaft only, with no elements on the outside of the shaft.

Section II. DERMATOPHYTES

5-6. INTRODUCTION

a. Dermatophytes commonly infect keratinaceous tissue such as hair, skin, and nails. This characteristic is thought to be due to an inhibitory agent in blood or serum that precludes establishment of infections at other body sites. While these organisms are pathogenic in man, they freely exist as soil saprophytes or zoopathogens. The infections are commonly referred to as "ringworm" due to round, serpentine lesions that appear to be caused by worms. The Latin word "tinea" which means "worm" is used to describe specific lesions.

b. The dermatophytes can be geophilic, zoophilic, or anthropophilic. The term geophilic is used for fungi whose natural habitat is in soil. Zoophilic refers to fungi that infect humans as well as lower animals. Anthropophilic means man-loving. Organisms in this category prefer to infect man.

5-7. MICROSPORUM SPP.

a. Within the genus, 11 species have been recognized. Direct examination of hair usually reveals an ectothrix infection in which fungal elements are found both inside and outside the hair shaft. Additionally, when the hyphae break up into arthroconidia they may demonstrate a "mosaic" pattern. Infected tissue will show septate hyphae with occasional branching. The organisms within this genus usually do not infect the nails. The microconidia are not characteristic enough to allow identification, so organisms are identified by their characteristic macroconidia. All are echinulated, meaning they have a "spiny" wall. They are also spindle-shaped with a varying cell wall thickness.

b. One of the characteristics used to differentiate organisms is the thickness of the walls separating the cells of the macroconidia. If the separating walls are thicker than the outside wall of the cell then the wall is considered thick-walled. If the separating walls of the macroconidia are thinner than the outer wall, the wall is considered thin-walled.

c. Microsporum audouinii.

(1) This fungus is found in the United States, Africa, and Europe. Colonies are slow growing and flat with short aerial hyphae. The surface color is gray or cream to tan. Reverse color is delicate peach or flesh. There is a band of color where slant and butt of culture media meet. A diagnostic feature is loss *of* characteristic color on SDA media within 14 days.

(2) Macroconidia are rarely seen microscopically. Microconidia, while seen, are non-diagnostic. Presence of sterile hyphae are often observed. The sterile rice grain test is used to distinguish Microsporum species (growth on the grains of rice) and Microsporum audouinii, no growth on the grains (figure 5-2).

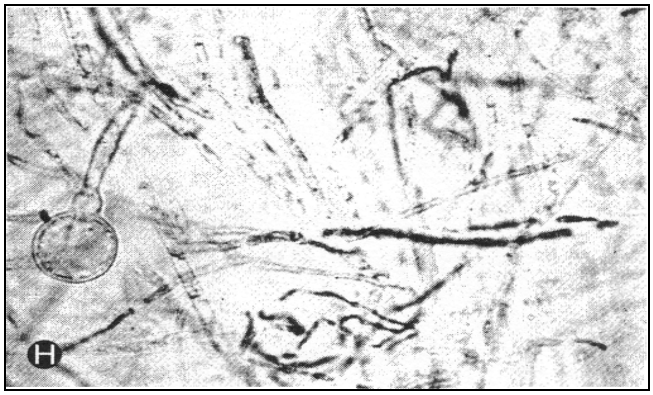


Figure 5-2. Microscopic morphology of Microsporum audouinii.

d. Microsporum canis.

(1) This organism was originally isolated from dogs. Today, it is isolated as frequently from cats and is distributed worldwide. Colonies are fast growing, initially white and fluffy, but becoming silky, with bright yellow pigment showing through the periphery. After two to four weeks the aerial mycelium is dense, cottony, tan, and sometimes in irregular tufts of concentric rings. The reverse is initially bright yellow, becoming buff orange-brown. Rare isolates show no pigment on reverse.

(2) Microscopic morphology shows microconidia that are small and non-diagnostic. The macroconidia, which are diagnostic, are usually numerous. They are truncated, thick-walled, and spindle-shaped with a "snout." Seven to fifteen cells are present in the macroconidia (figure 5-3). Microsporum canis may be confused with Microsporum audouinii, but may be differentiated by the sterile rice grain test.

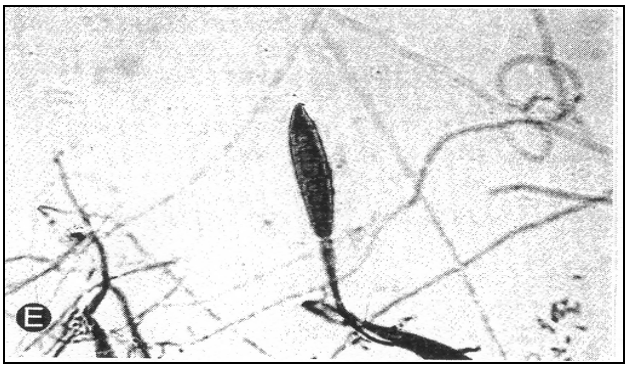


Figure 5-3. Microscopic morphology of Microsporum canis.

e. Microsporum gypseum

(1) Microsporum gypseum is a geophilic organism with worldwide distribution. The colonies grow rapidly with a flat, powdery surface colored various shades of tan. The reverse color ranges from yellow to slightly red.

(2) Microscopic morphology shows rare microconidia that are non-diagnostic. Macroconidia are numerous and diagnostic. They are wide, spindle-shaped with rounded ends. They contain three to six cells, with one end cut off and the other end rounded (figure 5-4).

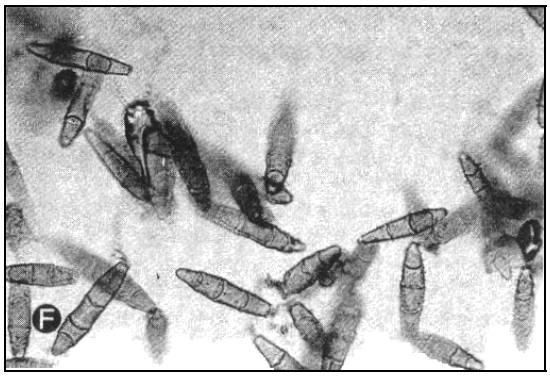


Figure 5-4. Microscopic morphology of Microsporum gypseum.

f. Microsporum nanum.

(1) Microsporum nanum is a zoophilic organism with worldwide distribution. The colonies are rapid growing and flat, with color ranging from cream to buff to cinnamon. Reverse color is splotchy brown.

(2) Microscopic morphology includes few microconidia that resemble an egg with the bottom cut off. Macro-conidia are numerous and diagnostic. They are pear-shaped with thick, rough outer walls, and contain one to three cells, most commonly two (figure 5-5).

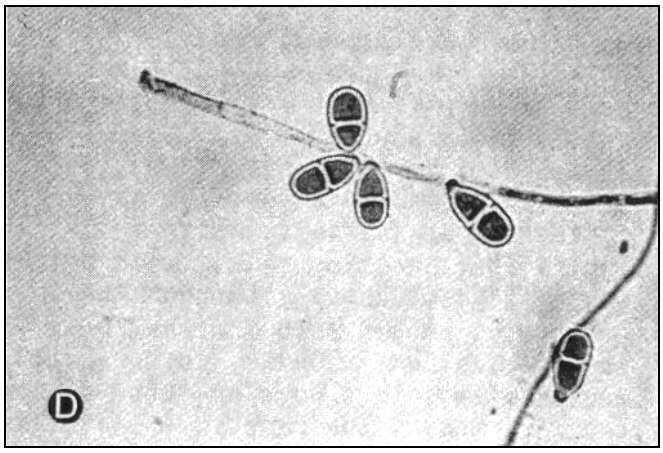


Figure 5-5. Microscopic morphology of Microsporum nanum.

5-8. EPIDERMOPHYTON FLOCCOSUM

a. Epidermophyton floccosum is the only pathogen within this genus. It is an anthropophilic organism with worldwide distribution. It infects skin and nails but does not infect hair. Colonial growth is slow, initially white and fluffy, becoming velvety and powdery. Color is khaki-green with a yellow border. Reverse color is non-characteristic. Surface is flat or radially folded.

b. Microscopic morphology shows no microconidia. Macroconidia are numerous and may be borne singly or in clusters of 2 or 3 on the same conidiophore. They have a blunt, "snow-shoe" shape with smooth, thin walls, and generally, three to four cells (figure 5-6).

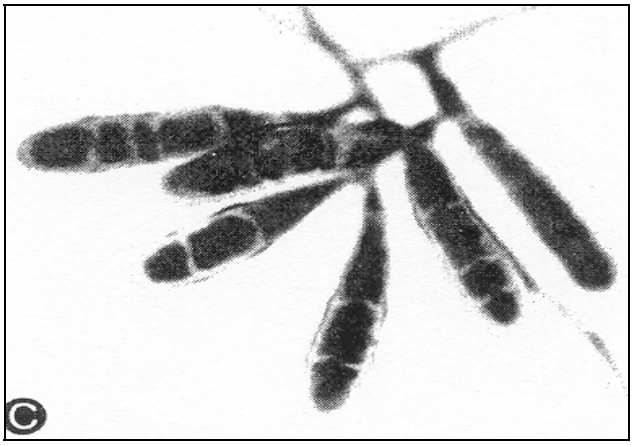


Figure 5-6. Microscopic morphology of Epidermophyton floccosum.

5-9. TRICHOPHYTON

a. There are 24 species within the genus. They can infect hair, skin, and nails. In this genus, the microconidia are diagnostic while the macroconidia are not.

b. There are two characteristic arrangements of the microconidia that will help distinguish this organism from similarly appearing genera: the "en thyrse" and "en grappe" arrangements. En grape refers to microconidia in clumps at the ends of the hyphae. En thyrse refers to microconidia that form along the sides of the hyphae.

c. Trichophyton mentagrophytes var. mentagrophytes.

(1) This organism is zoophilic with a worldwide geographic distribution. It infects hair, skin, and nails. Colonies are flat and granular with suede and red to tan granules. The reverse is buff to reddish-brown.

(2) Microscopic morphology shows macroconidia that are non-diagnostic. They may be club or pencil-shaped with thin, smooth walls. Microconidia are clavate (club-shaped) or globose, arranged "en thyrse" or "en grappe." Hyphae may form spirals (figure 5-7). Spiral hyphae, when present, are an identifying characteristic of this species. This organism produces a positive urease test within 4 days. The in vitro hair test is also positive. Microscopic observation of the in vitro hair test shows perpendicular invasion of the hair by this organism.

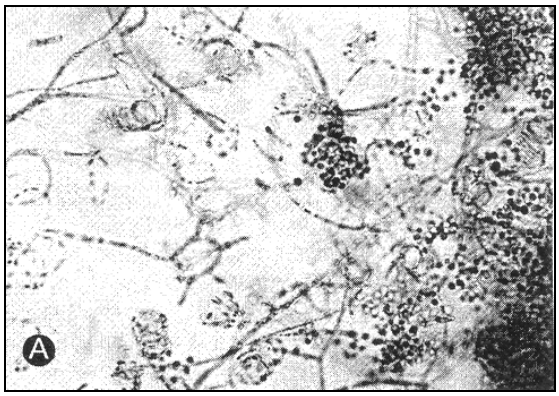


Figure 5-7. Microscopic morphology of Trichophyton mentagrophytes.

d. Trichophyton rubrum.

(1) This organism usually does not invade hair. Of many possible variants, seven are known in the United States. Infection by this organism serves as a marker for underlying diseases such as undiagnosed diabetes or leukemia. It is anthropophilic and found worldwide. Colonies are slow growing, flat or heaped at the center with a white, fluffy surface turning pink-tan. Reverse is wine-red.

(2) Microscopic morphology shows fragile, pencil-shaped macroconidia with smooth, thin walls. The microconidia are clavate, or globose, and "en thyrse". Microconidia are usually present in large numbers. The macroconidia are narrow with thin-walled parallel sides and contain two to eight cells. The confirmation tests, urease and in vitro hair tests, are both negative for this organism.

e. Trichophyton tonsurans.

(1) Trichophyton tonsurans is an anthropophilic organism that is distributed throughout the United States and Europe. It is a common cause of tinea capitis in both children and adults. Colonial growth is slow and may be of several types. All are markedly folded and appear as suede or granular. Colonies may be red, yellow, white, or other colors, with a deep orange to mahogany reverse.

(2) Microscopic morphology shows rare macroconidia that are cylindrical to clavate, slightly curved at the tops, and two to three-celled. Microconidia are extremely pleomorphic. Small microconidia tend to be globose to clavate and elongate, while large microconidia are globose resembling a balloon. A variety of shapes and sizes may be seen in the same culture. Growth is enhanced in a tube of medium containing thiamine.

f. Trichophyton verrucosum.

(1) Trichophyton verrucosum is a zoophilic organism with a worldwide distribution. Colonies are very slow growing. Initially they are small, heaped, glabrous, tough, and leathery. They appear white to yellowish tan, with a nonpigmented reverse.

(2) Microscopic morphology shows a typical absence of both microconidia and macroconidia. Chlamydospores produced form chains. This organism requires thiamine. Growth is enhanced in the presence of thiamine and inositol.

5-10. DISEASES CAUSED BY DERMATOPHYTES

a. **Tinea Pedis (Athlete's Foot)**. This is probably the most common and widely known dermatophytosis. It usually produces lesions between the toes, but may involve the entire foot. Lesions present as itchy, scaly, reddened areas in which dead epidermis and debris collect. Advanced lesions may develop purulent fluid and/or secondary bacterial infections. Causative agents are Trichophyton rubrum, Trichophyton mentagrophytes, and Epidermophyton floccosum.

b. **Tinea Manuum (Ringworm of the Hands)**. This is a dermatophytosis similar to tinea pedis but involving the hands. Infected areas are primarily the interdigital and palmar surfaces of the hands. Causative agents are same as tinea pedis.

c. **Tinea Cruris (Jock Itch)**. This dermatophytosis involves the groin, perineal, and perianal areas. Cause is usually due to sharing of contaminated clothing and/or towels. Lesions are dry, scaly, and itchy, with secondary infections possible. Causative agents are Microsporum canis, Trichophyton rubrum, and Epidermophyton floccosum.

d. **Tinea Corporis (Ringworm of the Body)**. This disease involves chiefly the glabrous skin, usually of the face, arms, and shoulders. It presents the classical "ringworm" type of lesion. The fungus is usually zoophilic or may be transmitted from an animal source. Trichophyton rubrum usually causes the most significant infections and, in diabetics, may cause a very resistant chronic infection. Causative agents are Microsporum canis, Trichophyton mentagrophytes, and Trichophyton rubrum.

e. **Tinea Barbae (Ringworm of the Beard)**. This disease is usually contracted from an animal vector and involves mainly the area of the face normally covered by facial hair (that is, eyebrows, beard). Trichophyton verrucosum is the leading cause of ringworm in cattle in the United States and is commonly acquired by dairymen. Infections of farmers due to Trichophyton mentagrophytes have been attributed to exposure to infected horses and dogs. Severe infections may produce serious or purulent discharges. Causative agents are Trichophyton verrucosum, Trichophyton mentagrophytes, and Trichophyton schoenleinii.

f. **Tinea Unguium (Ringworm of the Nails)**. This infection causes nails to become split, discolored, thickened, or crumbly and spongy. The disease is commonly found in conjunction with tinea pedis. Many so-called saprophytic fungi as well as Candida albicans can also be agents of this disease. Causative agents are Trichophyton rubrum and Trichophyton mentagrophytes.

g. **Tinea Capitis (Ringworm of the Scalp)**. This is a dermatophytosis of the scalp that usually presents as a dry, scaly lesion. The infection is most common in children with Trichophyton tonsurans as the most common etiological agent. While differentiation of the etiological agents requires expertise, all Microsporum spp., except Microsporum gypseum, are readily diagnosed by the production of a yellow/green fluorescence of infected hairs when illuminated by a Wood's lamp. Causative agents are Microsporum canis, Microsporum audouinii, Microsporum distortum, Trichophyton schoenleinii, Trichophyton tonsurans, and Trichophyton violaceum.

Continue with Exercises

EXERCISES, LESSON 5

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement or by writing the answer in the space provided.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. What disease is caused by Malassezia furfur? _____

2. Laboratory identification of Malassezia furfur is most often made by:

   a. Direct examination of infected hairs.

   b. Observation of colonial growth.

   c. Direct examination of skin scrapings.

   d. Examination of microscopic colonial morphology.

3. Which organism is the cause of tinea nigra?

   a. Piedra hortae.

   b. Exophiala werneckii.

   c. Malassezla furfur.

   d. Trichophyton rubrum.

4. What color are colonies of Exophiala werneckii?

   a. Beige.

   b. Dark green.

   c. Light brown.

   d. Black.

5. If a patient reports a metallic sound upon combing hair, what organism might be the infective agent?

   a. Piedra hortae.

   b. Trichosporon beigelii.

   c. Exophiala werneckii.

   d. Malassezia furfur.

6. What two characteristics aid in discriminating Trichosporon beigelli from Geotrichum candidum?

   _____

   _____

7. Infections by dermatophytes are erroneously called _____ infections.

   a. Cutaneous.

   b. Keratinaceous.

   c. Ringworm.

   d. Ectothrix.

8. Hyphae which break up into a mosaic pattern of arthroconidia are characteristic of:

   a. Trichosporon beigelii.

   b. Microsporum spp.

   c. Trichophyton spp.

   d. Tinea capitis.

9. List the identifying characteristics of Microsporum macroconidia.

_____

_____

_____

10. Microsporum audouinii differs from other Microsporum spp. in:

    a. The microscopic appearance of its macroconidia.

    b. The results of the sterile rice grain test.

    c. The appearance of domed terminal vesicles.

    d. None of the above.

11. The presence *of* macroconidia which are numerous and spindle-shaped with a "snout" suggests infection with:

    a. Microsporum canis.

    b. Microsporum nanum.

    c. Epidermophyton floccosum.

    d. Trichophyton spp.

12. Colonies of Microsporum gypseum have a reverse color that is:

    a. Brown.

    b. Black.

    c. Peach.

    d. Yellow.

13. Epidermophyton floccosum is likely to be isolated from specimens.

    a. Hair or nails.

    b. Skin, hair, or nails.

    c. Skin or nails.

    d. Skin or hair.

14. Laboratory indicators of the presence of Trichophyton mentagrophytes include:

    _____

    _____

    _____

15. An organism that can serve as a marker for other underlying disease is:

    a. Trichophyton tonsurans.

    b. Trichophyton rubrum.

    c. Trichophyton mentagrophytes.

    d. Epidermophyton floccosum.

16. An anthropophilic organism commonly found in tinea capitis infections is:

    a. Trichophyton tonsurans.

    b. Trichophyton rubrum.

    c. Trichophyton verrucosum.

    d. Trichophyton mentagrophytes.

17. Dermatophytes that commonly cause athlete's foot include:

    a. Trichophyton verrucosum.

    b. Trichosporon beigelli.

    c. Epidermophyton floccosum.

    d. Microsporum canis.

18. Fungi that cause "ringworm" infections are frequently transmitted by:

    a. Contaminated soil.

    b. Air droplets.

    c. Animals.

    d. Human carriers.

19. If a hair specimen taken from a patient with scalp ringworm displays a yellow-green fluorescence under a Wood's lamp, it is likely to be:

    a. Any member of Microsporum spp.

    b. Any member of Trichophyton spp.

    c. A member of Trichosporon spp.

    d. A member of Microsporum spp.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 5

1. Tinea versicolor. (para 5-2a)

2. c (para 5-2b)

3. b (para 5-3a)

4. d (para 5-3b)

5. a (para 5-4a)

6. Blastoconidia.
   Pseudohyphae. (para 5-5b)

7. c (para 5-6a)

8. b (para 5-7)

9. Echinulated.
   Spindle-shaped.
   Varying cell wall thickness. (para 5-7a)

10. b (para 5-7c(2))

11. a (para 5-7d(2))

12. d (para 5-7e(1))

13. c (para 5-8a)

14. Spiral hyphae.
    Positive urease test.
    Perpendicular invasion of hair. (para 5-9c(2))

15. b (para 5-9d(1))

16. a (para 5-9e(1))

17. c (para 5-10a)

18. c (paras 5-10d, e)

19. d (para 5-10g)

End of Lesson 5

LESSON ASSIGNMENT

LESSON 6	Subcutaneous Fungi.
LESSON ASSIGNMENT	Paragraph 6-1 through 6-5.
LESSON OBJECTIVES	Upon completion of this lesson, you should be able to:

6-1. Select the statement that correctly describes the specific fungus that causes mycetoma.

6-2. Select the statement that correctly describes the specific fungus that causes chromomycosis.

6-3. Select the statement that correctly describes the specific fungus that causes rhinosporidiosis.

6-4. Select the statement that correctly describes the specific fungus that causes sporotrichosis.

SUGGESTION After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 6

SUBCUTANEOUS FUNGI

6-1. INTRODUCTION

Subcutaneous fungi cause mycoses that involve the skin and subcutaneous tissue. Dissemination to the major organs of the body usually does not occur. However, involvement of large muscle mass and bone may develop. They are primarily soil organisms found in tropic and subtropic regions. Man, acting as an accidental host, becomes infected either by traumatic implantation of the organism or by the introduction of viable spores into a lesion. Subcutaneous disease processes include mycetomas, chromomycosis, rhinosporidiosis, and sporotrichosis.

6-2. MYCETOMA

a. Mycetoma is a chronic, progressive disease characterized by tumor-like swelling, abscess, and draining sinus tracts. Fluids that ooze from these tracts contain pigmented granules composed of microbial elements. The causative agents can be either bacterial or fungal. When bacteria are the cause of the disease process, three genera are usually suspected: Actinomyces, Nocardia, or Streptomyces. Bacterial mycetomas, caused by anyone of these genera, are called actinomycotic mycetomas. Other bacteria may cause similar disease process called botryomycosis. The term eumycotic mycetoma is applied when the disease is caused by a fungus. Eumycotic mycetoma may also be referred to as Maduromycosis, and is often called "Madura foot." This name evolved because the disease, which causes primary infections of the foot, was first described in the Madura province of India.

b. Eumycotic mycetoma is usually resistant to chemotherapy. Definitive treatment may require amputation. There are several possible causative agents of which Pseudallescheria boydii is the most common. Other causative agents are Acremonium falciform, Exophiala jeanselmei, Madurella grisea, and Madurella mycetomatis. Recovery of the causative agent is obtained by culturing the pigmented granules. The granules range in color from yellowish to red and black. Color is dependent on the causative agent. Upon direct examination of a crushed granule, mycelial elements can be observed under the microscope. Remaining granules should be washed in sterile water or antibiotic solution and inoculated to SDA and SDA with chloramphenicol.

(1) Pseudallescheria boydii (Monosporium apiospermum: asexual).

(a) The granules produced by Pseudallescheria boydii are white to yellow and soft to firm. Colonies are fluffy in texture and grayish to white color. Reverse is grayish-black.

(b) Microscopic examination of colonies shows hyphae that are hyaline, broad, and septate. Conidia are numerous, pyriform to oval, small (6 to 9 mcm) and hyaline, or large and brown to golden. They are found as single cells or in bunches on a short conidiophore (figure 6-1).

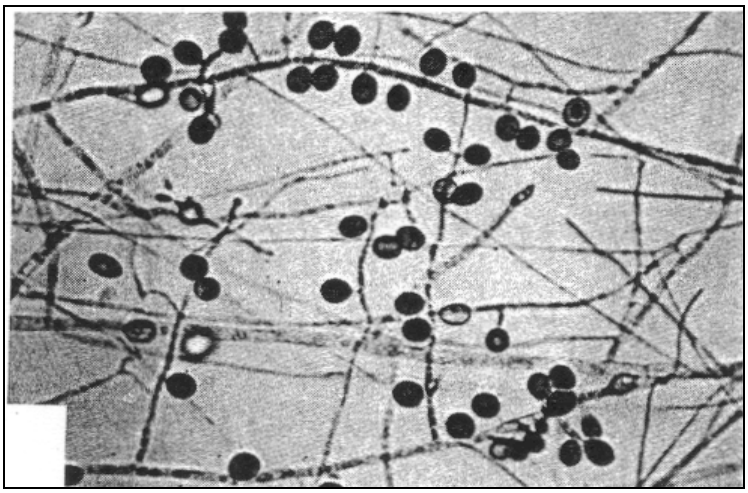


Figure 6-1. Microscopic morphology of Pseudallescheria boydii.

(2) Exophiala jeanselmei.

(a) Exophiala jeanselmei, the second most common cause of Madura foot, produces dark granules in host tissue. They are brown to black, irregular in shape, and fragile. Detached portions or fragments often are found in the lesion, within giant cells. Colonies are slow-growing on culture media and produce a black color with a velvety texture. The reverse is also black.

(b) Microscopic examination shows hyphae that are dematiaceous and septate. Young cultures appear as yeast-like budding cells. Eventually septate hyphae with conidiophores develop. Annellides are cylindrical, forming oval to oblong annelloconidia. The annelloconidia are loosely clustered at the tip of the annellides, and may be seen laterally along the hyphae.

(3) Acremonium falciform.

(a) Granules of Acremonium falciform are soft, and white to pale yellow. Colonies are slow-growing on culture media, producing color that is buff to lavender and a texture that is downy. Reverse is currant red to violet.

(b) Microscopic examination shows delicate septate hyphae. Tapering phialides rise directly from the hyphae and produce phialoconidia. These phialoconidia are hyaline, curved, and rounded on the upper end with a straight base. They are arranged in a ball at the tip of the phialide because they are held together with a mucin-like substance (figure 6-2).

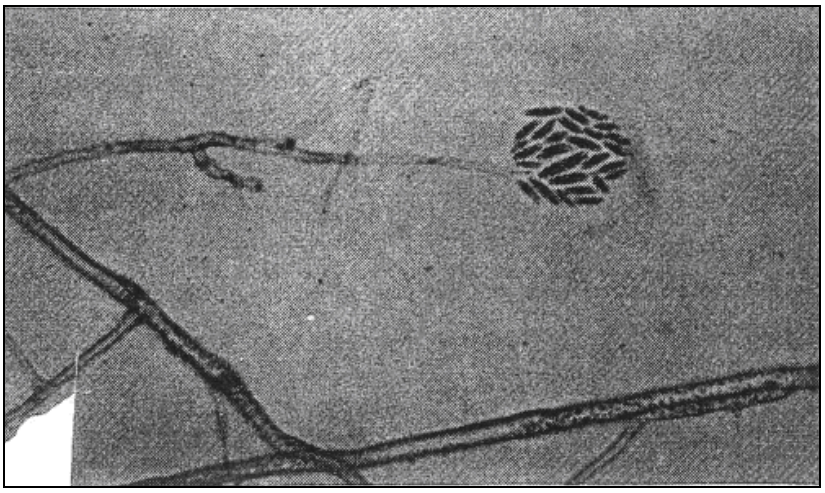


Figure 6-2. Microscopic morphology of Acremonium falciform.

6-3. CHROMOMYCOSIS

a. Chromomycosis is a disease that affects the legs and feet. The causative agents are termed dematiaceous because they have a brown to black pigment due to the presence of melanin. These organisms are often referred to as "black molds." They are slow growing, geophilic fungi that belong to the class Deuteromycetes. Causative agents include Cladosporium carrionii, Phialophora verrucosa, Fonsecaea pedrosoi, and, rarely, Fonsecaea compacta.

b. Clinical symptoms vary depending on the age of the infection. Early lesions begin as small erythematous papules which satellite around the periphery of the primary lesion. As the infection progresses, the lesions aggregate. become red to violet, and ulcerate. Older lesions are described as "cauliflower-like." These lesions are dry, crusted, verrucous (rough or warty in appearance), and are darkly pigmented with defined margins. Complications often develop with bacterial superinfections occurring within the lesions.

c. Specimens include exudates and scrapings. Sclerotic bodies are observed upon direct microscopic examination. Regardless of the causative agent, sclerotic bodies appear to be round, thick-walled, 6 to 12 mcm in size, and chestnut brown in color. Because of their color and shape, they are commonly referred to as "copper pennies," although it is believed they are nothing more than chlamydospores.

d. Specimens are inoculated to SDA and Mycosel™. The causative agents usually require three weeks incubation for growth to occur. Because of the time needed for initial growth, a negative report may not be sent out until there is no growth at the end of six weeks incubation.

(1) Cladosporium carrionii vs. Cladosporium spp.

(a) Colonies of Cladosporium carrionii are rapid-growing glabrous to velvety in texture, and produce a color that is gray-green to olive-black. The reverse color is black.

(b) Microscopic examination reveals branching chains of blastoconidia that are borne on a dematiaceous conidiophore. Microconidia are smooth to slightly echinulate, and range in color from pale to dark brown. Conidia closest to the conidiophore, where chains branch, are referred to as shield cells because of their shape. Distinguishing Cladosporium carrionii from other members of the genus requires either a Loeffler's serum slant liquefaction or a gelatinase test. In both tests, cladosporium carrionii is negative while other members of the genus Cladosporium are positive (figure 6-3).

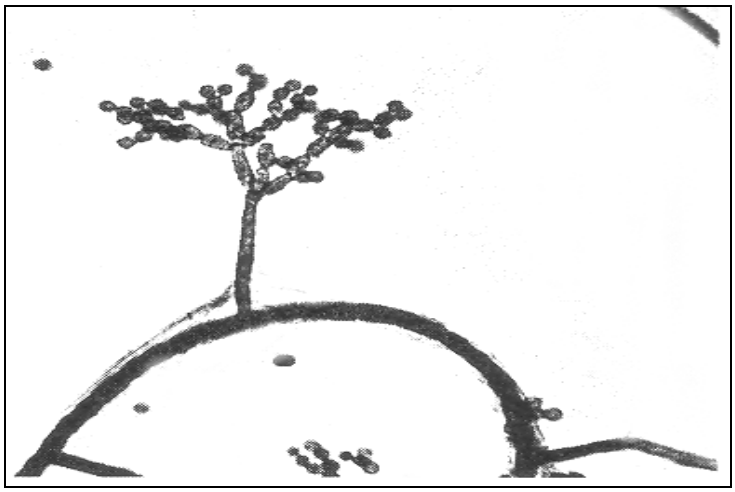


Figure 6-3. Microscopic morphology of Cladosporium carrionii.

(2) Phialophora verrucosa.

(a) Phialophora colonies are rapid growing, with a dark olive-gray to black color, and a flat to velvety texture. Reverse color is black.

(b) Microscopic examination reveals darkly pigmented, vase-shaped phialides, with a flared cup-shaped collarette. Phialoconidia are ovoid to ellipsoidal and hyaline with basal scar. Due to a mucin-like substance, the micronidia usually remain clustered at the apex of the phialide, resembling a bouquet of flowers in a vase (figure 6-4).

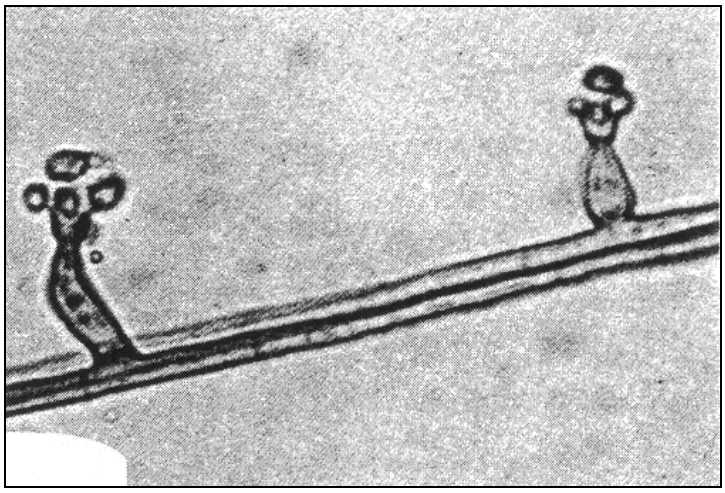


Figure 6-4. Microscopic morphology of Phialophora verrucosa.

(3) Fonsecaea pedrosoi.

(a) Fonsecaea pedrosoi colonies are slow growing, and velvety to fluffy, with a color that is black to brown-tinged as colonies age. Reverse color is black.

(b) Microscopic examination can show three types of sporulation that may be exhibited by this organism. For proper identification, at least two of the three types must be seen within the same culture. "Phialophora-type" sporulation shows vase-shaped phialides with flared cup-shaped collarettes. Phialoconidia are clustered at the tip of the phialides, as seen in Phialophora verrucosa. "Cladosporium-type" sporulation shows slender conidiophores that bear branched micronidia similar to the genus Cladosporium. "Rhinocladiella-type" sporulation shows oval microconidia formed along the top and sides of the conidiophore (similar to Figure 6-5.)

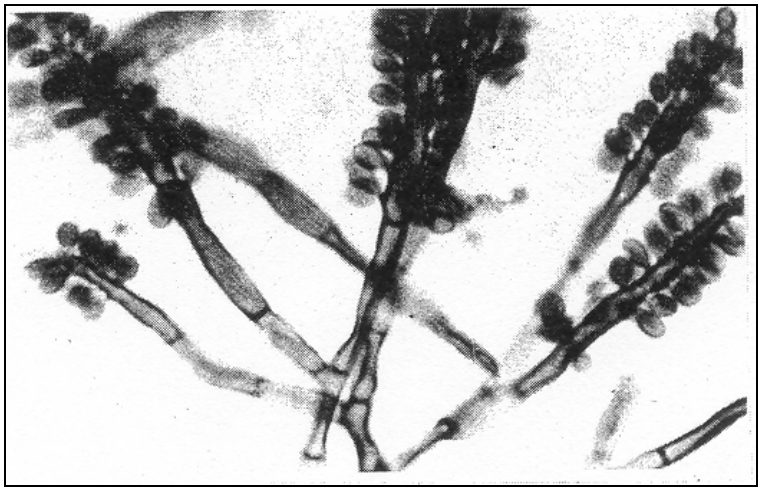


Figure 6-5. Microscopic morphology of Fonsecaea pedrosoi.

(4) Fonsecaea compacta.

(a) Fonsecaea compacta is rarely isolated. When grown on culture media, colonies are dark olive to black, with a heaped, brittle texture. Reverse is black.

(b) Microscopic examination shows morphology similar to Fonsecaea pedrosoi, except that conidia are round.

6-4. RHINOSPORIDIOSIS

a. Rhinosporidiosis is a mycotic infection of the mucous membranes characterized by development of polyps. Symptoms vary, depending upon the stage of tumor development and site infected. Polyps are usually pink to purple and brittle. Other symptoms include hyperplasia of mucous membranes and granulation tissue with scarring. Geographical distribution includes India and Ceylon. The source of infection is assumed to be water. Causative agent is Rhinosporidium seeberi.

b. Direct examination of specimens show thick-walled and refractile spherules, 40 to 300 mcm in size. Endospores contained within the spherules are 6 to 8 mcm in size. Culture of the organism is usually not successful.

6-5. SPOROTHRICHOSIS

a. Sporothrichosis is a disease process caused by only one etiological agent (Sporothrix schenckii.) The primary lesion, usually on a finger, begins as a small movable, nontender, subcutaneous nodule that slowly enlarges, adheres to the skin, becomes pink and later necrotic, and finally ulcerates. In a few days or weeks, similarly discolored subcutaneous nodules appear along the course of the draining lymphatic tract.

b. Clinical specimens may include skin scrapings, pus, and biopsy material. Use of a periodic acid-Schiff stain for direct examination reveals small budding blastoconidia ("cigar bodies"), elongated, non-budding blastoconidia, and possible a basophilic staining yeast cell surrounded by an eosinophilic covering consisting of a precipitated antibody-antigen complex ("asteroid bodies").

c. Specimens are inoculated to SDA and SDA with cycloheximide. Sporothrix schenckii is a diphasic organism. When an inoculated BHIA is incubated at 35º C, the yeast phase is seen. Microscopically, elongated, budding blastoconidia are observed. These are the same structures seen under direct examination. When cultured and incubated at 25º C, the mold phase is recovered. Growth is obtained in 3 to 7 days. Color is white to tan, with a glabrous texture.

d. Microscopic examination shows finely branching, septate hyphae. Small microconidia are arranged along the sides of the hyphae giving an "en thyrse" formation, but are more characteristically seen bunched on a common conidiophore in a "flowerette" formation. Clinical symptoms along with isolation and identification of the organism Sporothrix schenckii in a culture is diagnostic (figure 6-6).

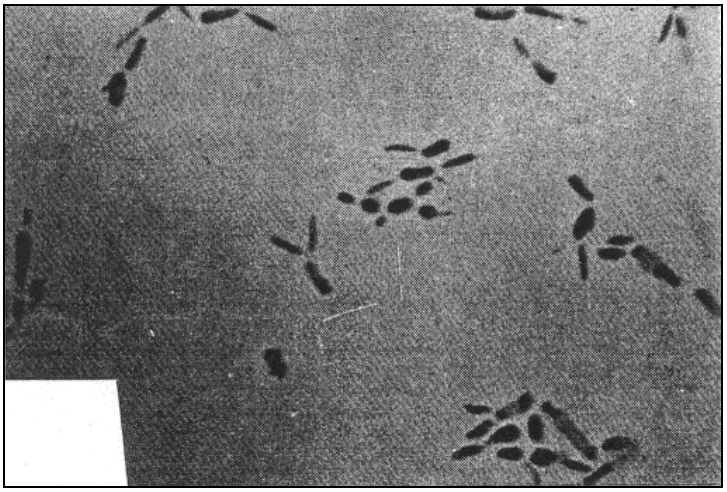


Figure 6-6. Microscopic morphology of Sporothrix schenckii.

Continue with Exercises

EXERCISES, LESSON 6

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. List three disease processes that can be caused by subcutaneous fungi.

   _____

   _____

   _____

2. "Madura foot" is the common term used to refer to eumycotic mycetoma infections of the foot.

   a. True.

   b. False.

3. What organism is the most common cause of "Madura foot"?

   a. Actinomyces.

   b. Pseudallescheria boydii.

   c. Exophiala jeanselmei.

   d. Streptomyces.

4. Granules produced by Exophiala jeanselmei are:

    a. White to yellow in color.

    b. Found as fragments in a lesion.

    c. Caused by bacterial contamination.

    d. Consistent in shape.

5. Microscopic examination of Acremonium falciform shows:

    a. Conidia held together by a mucin-like substance.

    b. Septate hyphae.

    c. Phialoconidia that are curved.

    d. All of the above.

6. Chromomycosis is a disease process caused by:

    a. Dematiaceous fungi.

    b. Cutaneous fungi.

    c. Anthropophilic fungi.

    d. Eumycotic fungi.

7. List two genera that cause chromomycosis.

    _____

    _____

8. Cladosporium carrionii differs from other members of the genus in which of the following ways?

   a. Production of conidia referred to as spear cells.

   b. Negative reaction to gelatinase and Loeffler's serum slant liquefaction tests.

   c. Negative reaction to gelatinase, positive to Loeffler's serum slant liquefaction tests.

   d. Positive reaction to gelatinase test only.

9. Identification of phialoconidia that are ovoid to ellipsoidal, and hyaline with basal scar, suggests the presence of which of the following organisms?

   a. Cladosporium carrionii.

   b. Exophiala jeanselmei.

   c. Phialophora verrucosa.

   d. Rhinosporidium seeberi.

10. Which three types of sporulation are diagnostic of Fonsecaea pedrosoi?

    _____

    _____

    _____

11. Rhinosporidiosis is defined as a mycotic infection of:

    a. Mucous membranes.

    b. Legs and feet.

    c. Hands and fingers.

    d. Large muscle mass.

12. Which of the following body areas is most likely to be infected by Sporothrix schenckii?

    a. Scalp.

    b. foot.

    b. Intestinal tract.

    c. Lymphatic tract.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 6

1. Any three of the following:
   Mycetoma.
   Chromomycosis.
   Rhinosporidiosis.
   Sporotrichosis. (para 6-1)

2. a (para 6-2a)

3. b (para 6-2b)

4. b (para 6-2b(2)(a))

5. . (para 6-2b(3)(b))

6. a (para 6-3a)

7. Any two of the following:
   Cladosporium.
   Phialophora.
   Fonsecaea. (para 6-3a)

8. b (para 6-3d(1)(b))

9. c (para 6-3d(2)(b))

10. Phialophora-type.
    Cladosporium-type.
    Rhinocladiella-type. (para 6-3d(3)(b))

11. a (para 6-4a)

12. d (para 6-5a)

End of Lesson 6

LESSON ASSIGNMENT

LESSON 7	Systemic Mycoses.
TEXT ASSIGNMENT	Paragraphs 7-1 through 7-5.
TASK OBJECTIVES	After completing this lesson, you should be able to:

7-1. Select the statement that correctly describes characteristics of Histoplasma capsulatum.

7-2. Select the statement that correctly describes characteristics of Blastomyces dermatitidis.

7-3. Select the statement that correctly describes characteristics of paracoccidioides brasiliensis.

7-4. Select the statement that correctly describes characteristics of Coccidioides immitis.

SUGGESTION After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 7

SYSTEMIC MYCOSES

7-1. INTRODUCTION

a. As a group, systemic mycoses usually originate as pulmonary system infections. They may progress systemically or affect any internal organ and may show cutaneous and subcutaneous involvement. Infections may vary from mild, flu-like respiratory syndromes to severely fulminating systemic infections. Organisms causing systemic mycoses are exogenous and are acquired via inhalation of viable spores. With the exception of Cryptococcus neoformans, all are diphasic, having two different morphological forms that are temperature dependent. Clinical specimens include skin scrapings, sputum, and biopsy material.

NOTE: Because of their extreme pathogenicity, it is of extreme importance that any specimen likely to contain a systemic organism be handled with caution and only within a biological safety hood.

b. The systemic mycoses are histoplasmosis, blastomycosis, paracoccidioidomycosis, coccidioidomycosis, and cryptococcosis (see Lesson 4).

7-2. HISTOPLASMOSIS

a. Histoplasmosis is a disease caused by Histoplasma capsulatum or by Histoplasma duboisii. These organisms are found in soil that is fertilized by the droppings of fowl (especially chickens and blackbirds) and bats. Histoplasma capsulatum causes North American histoplasmosis and is endemic to areas of the United States such as the central Mississippi River valley, the Ohio River valley, and the Appalachian Mountains. Histoplasma duboisii causes African histoplasmosis and is endemic to central and western Africa.

b. Primary infection occurs by inhalation of viable spores. In the initial infection, lesions with lymph-node enlargement occur throughout the lung. Majority of cases are asymptomatic, or present as self-limiting mild respiratory infections. Positive skin tests and calcifications in the lung may be incidental findings long after the infection has resolved. However, some infections cause a large variety of clinical manifestations because of tendency to enter the bloodstream during the primary infection and cause progressive disease at one or more anatomic sites. If this occurs, calcified lesions are seen in the affected organs and tissue. The body attempts to clear the fungus from the bloodstream via the reticuloendothelial cells located throughout the body. These cells are always involved in a systemic infection.

c. Diagnosis of histoplasmosis depends on the stage of the mycosis. Screening for acute pulmonary histoplasmosis may be accomplished with a positive skin test using histoplasmin, a mycelial antigen. The skin test may show a positive reaction as early as two weeks after exposure and will then remain positive for years. Serological titers against yeast and mycelial antigens are performed for a positive skin test. A single serum titer of equal to or greater than 16 against the yeast antigen is highly suggestive of recent infection. A rise in titer between sequentially collected sera is diagnostic of a current infection. In disseminated histoplasmosis, skin tests and the complement fixation titers are negative in more than half the cases. Organisms may be seen on direct blood smears and may be grown from routine blood cultures if incubated for 10 to 15 days. The organism may also be seen on smears from bone marrow aspirates, liver biopsy, and skin lesions. Urine cultures may be positive. All tissue obtained for fungal studies should be stained with Gomori's methenamine, silver, or periodic acid-Schiff stains.

d. Laboratory identification of Histoplasma.

(1) Direct examination of clinical specimens shows single, budding blastoconidia. H. capsulatum is 1 to 5 mcm in size while H. duboisii is 7 to 15 mcm. Organisms growing intracellularly in mononuclear cells is a diagnostic feature. H. capsulatum is oval with a vacuole and dark staining crescent body. H. duboisii is thick-walled and oval to hourglass in shape.

(2) Mycelial forms of H. capsulatum and H. duboisii are indistinguishable. Colonial growth rate is slow, taking 10 to 14 days. Surface color of colonies is white to tan, with reverse being non-pigmented to orange; appearance is cottony. Microscopic examination reveals branching, septate hyphae. Small microconidia (2 to 5 mcm) are pyriform to round and borne on short conidiophores. Large microconidia (7 to 25 mcm) are tuberculate, thick-walled, round to pyriform, and borne on short conidiophores. (figure 7-1.)

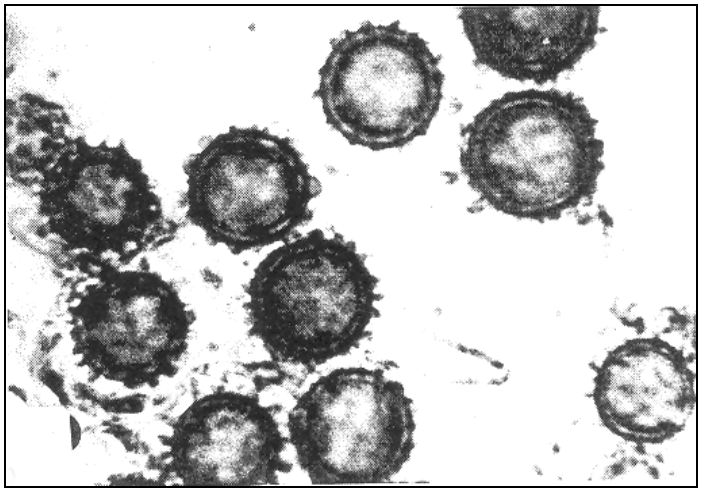


Figure 7-1. Mycelial form of Histoplasma capsulatum.

(3) Care must be taken not to confuse the mold phase of Histoplasma capsulatum with Sepedonium species. This saprophyte resides in soil and Histoplasma capsulatum may be recovered from sputum as a contaminant. In vitro conversion of the mold phase to yeast phase and back to mold phase of Histoplasma capsulatum will confirm its identification.

(4) The yeast phase of Histoplasma requires a temperature of 35º C. The growth rate is slow, taking 5 to 10 days for colonial growth. Colony color is white to cream with a butyrous texture. Microscopic morphology shows oval budding blastoconidia. H. capsulatum is small, 2 to 3 mcm in diameter (figure 7-2.) H. duboisii are large, 8 to 12 mcm in size.

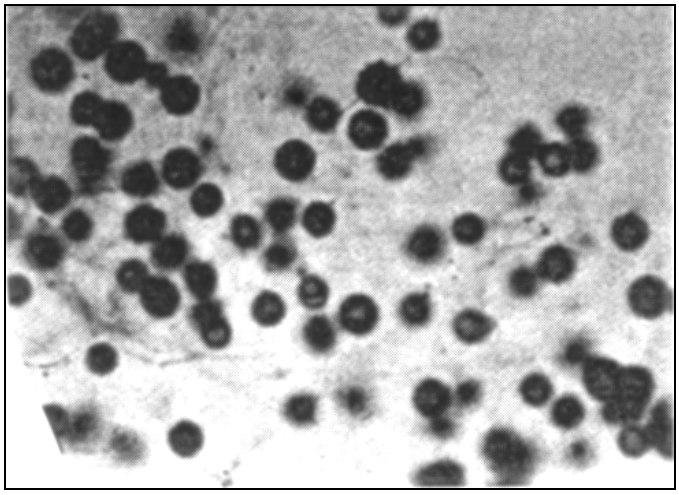


Figure 7-2. Yeast of Histoplasma capsulatum.

7-3. BLASTOMYCOSIS

a. The etiological agent of blastomycosis (North American blastomycosis) is Blastomyces dermatitidis. It occurs most often in individuals who come in close contact with soil, especially in the Mississippi and Ohio River valleys.

b. Primary infection of the lungs occurs upon inhalation of viable spores from the soil. Because of a strong, natural resistance to this organism, infections are often asymptomatic and are detected only by a positive skin test. In typical cases of progressive blastomycosis, the patient experiences symptoms similar to a persistent "chest cold" with low-grade fever, weight loss, and progressive disability. Examination reveals evidence of pneumonia that may involve any segment or lobe of the lung. Cavity formation in the lung and lymph node involvement are frequent. Pulmonary lesions may heal while lesions of skin, bone, and joints continue to develop. As these later lesions form, symptoms, such as fever, sweats, chills, and weakness increase. Untreated patients with systemic blastomycosis may die as early as six months but more commonly in one to two years post infection.

c. Laboratory identification of Blastomyces dermatitidis.

(1) Clinical specimens include sputum, skin scrapings, pus, and biopsy material. Complement fixation tests are of little value because antigens are neither specific nor sensitive. Immunodiffusion is specific with a sensitivity of 80 percent. Diagnosis is usually made by recovery of organism. During yeast phase, this organism is white to tan in color with a verrucose texture. Microscopic examination of clinical material reveals yeast form of the organism that is characterized by thick-walled blastoconidia (8 to 15 mcm in diameter.) Single buds are attached to the parent cell by a broad base (figure 7-3.)

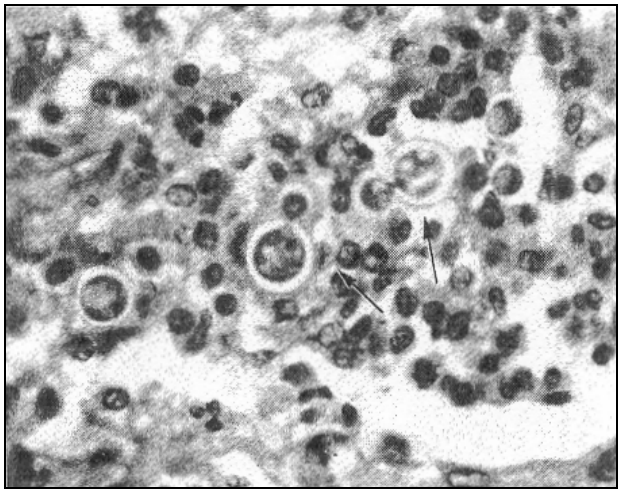


Figure 7-3. Yeast form of Blastomyces dermatitidis.

(2) The mycelial phase of Blastomyces dermatitidis exhibits a slow growth rate of 7 to 14 days. Colonies are white to tan, with a cottony appearance. Microscopic examination shows branching, septate hyphae. Microconidia are 4 to 5 mcm, pyriform, and located terminally on short conidiophores. They are often referred to as "lollipops " (figure 7-4.)

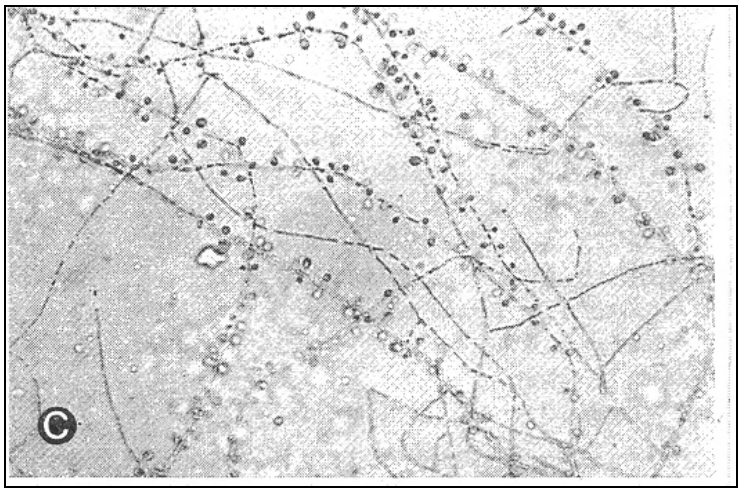


Figure 7-4. Mycelial form of Blastomyces dermatitidis.

7-4. PARACOCCIDIOIDOMYCOSIS

a. Paracoccidioidomycosis (commonly called South American blastomycosis) is a disease process caused by Paracoccidioides brasiliensis. Primary inoculation is by inhalation of viable spores into the lungs. The disease rarely progresses, disseminates, or causes severe infection if immunity is normal. There is a marked loss of pulmonary function however when it does occur. The primary lesions usually heal and remain latent but may be reactivated years later. Reactivation may occur for no apparent reason or may be secondary to immunosuppressive disease or therapy. Upon reactivation, lesions progress and disseminate to other organs. Mucocutaneous ulcers are the most common disseminated manifestation. These ulcers originate as papules, develop into vesicles, and later become encrusted, granulomatous ulcers. Deeper destruction with spreading to adjacent tissue follows. Regional lymph nodes ulcerate and develop draining sinuses. Dissemination continues in the lymphatic system, spleen, intestine, and liver.

b. Diagnosis is based on the demonstration of paracoccidioides brasiliensis in specimens taken from lesions. Clinical specimens include scraping of mucocutaneous lesions, exudate, pus, sputum, bronchial washings, feces, and tissue taken by biopsy. Direct examination of clinical specimens demonstrates the yeast phase of this diphasic organism that is identified by thick-walled globose blastoconidia (ranging in size from 12 to 14 mcm.) It has multiple buds attached to the parent cell by thin necks. Buds are 2 to 5 mcm in size. This form is often referred to as the "pilot's wheel" (figure 7-5.)

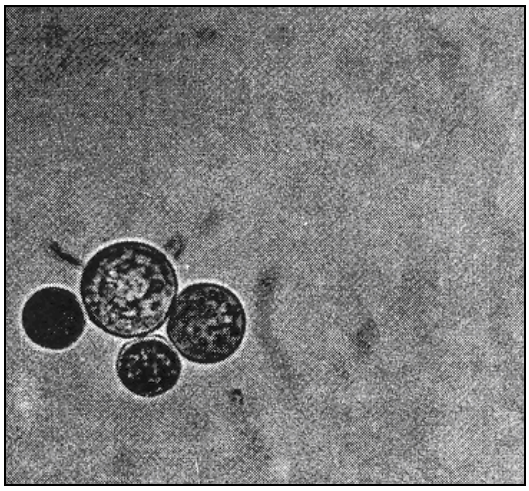


Figure 7-5. Yeast form of Paracoccidioides brasiliensis.

c. Laboratory Identification of paracoccidioides brasiliensis.

(1) The mycelial phase of Paracoccidioides brasiliensis is slow growing, taking 14 to 21 days for visible growth. Colonies are white to tan, with a texture that is downy to heaped. Microscopic examination shows fine, branching, septate and usually sterile hyphae, but will occasionally show sessile microconidia on the hyphae (figure 7-5.)

(2) During the yeast phase of this organism, colonies are white to tan with a verrucose texture. Macroscopic colony morphology resembles Blastomyces dermatitidis.

7-5. COCCIDIOIDOMYCOSIS

a. The etiological agent for coccidioidomycosis (commonly called Valley Fever or San Joaquin Valley Fever) is Coccidioides immitis. Geographic distribution is the lower Sonoran life zone of the US, the desert region of Mexico, and Central and South America where soil is dry, arid, and alkaline. The organism does not survive in moist climates because of competing fungal flora.

b. Coccidioides immitis is diphasic. Instead of having the usual yeast and mold phase, it has a mold phase at room temperature and a tissue phase in vivo. Because it has two morphologically different phases that are temperature dependent, it qualifies as a diphasic or dimorphic organism. It resides in the soil as mycelia composed of hyphae that are 2 to 4 mcm in diameter. Hyphae fragment into highly resistant arthroconidia. Arthroconidia become airborne whenever soil is disturbed. Arthroconidia are inhaled by humans, initiating the infection. Each arthroconidium develops into a spherule (tissue phase) in vivo. Each spherule, 36 to 60 mcm in diameter, contains numerous endospores that are 2 to 5 mcm in diameter. Endospores are released when the spherules rupture, spreading the infection within the host.

c. Coccidioidomycosis may present as acute to subacute pneumonia that may become complicated by chronic pulmonary infection, or disseminate to other body organs. 60 percent of reported infections are asymptomatic, whereas 40 percent demonstrate fever, a mild cough, and a small amount of white sputum. Caucasians may develop a rash due to allergic reaction. When primary, the pulmonary infection fails to heal. Dissemination occurs via the bloodstream. In untreated patients, mortality rate is 100 percent if the central nervous system becomes involved. Other infective sites are lung, liver, bone, spine, joints, and subcutaneous tissue. Only a small percentage of infections progress past the primary pulmonary stage.

d. Laboratory identification of Coccidioides immitis.

(1) Diagnosis is made by spherule demonstration in the direct examination of clinical specimens. Culturing can be accomplished. However arthroconidia seen in culture (figure 7-6) are highly infective and should be handled only in biological safety hoods.

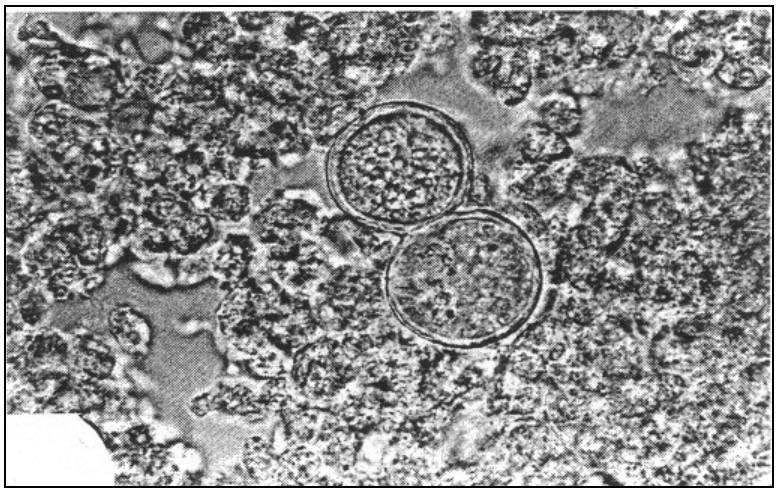


Figure 7-6. Spherule of Coccidioides immitis in tissue.

(2) Mycelial phase of C. immitis exhibits a moderate growth rate of 5 to 14 days. Colonies are white to tan with a brown to black reverse. Appearance is cottony. Microscopic examination shows branching septate hyphae. Rectangular to barrel shaped arthroconidia range in size from 3 to 5 mcm. Alternating light and dark staining cells are present (figure 7-7.) No yeast phase occurs.

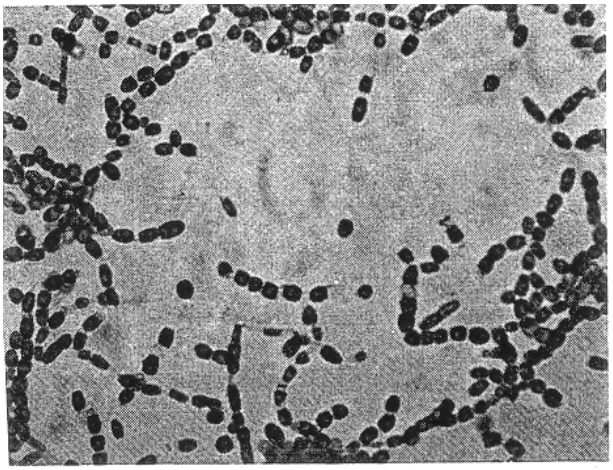


Figure 7-7. Mycelial form of Coccidioides immitis.

Continue with Exercises

EXERCISES, LESSON 7.

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. What is the usual mode of infection for the systemic fungi?

   _____

2. Which of the following systemic fungus is NOT diphasic?

   a. Cryptococcus neoformans.

   b. Histoplasma capsulatum.

   c. Blastomyces dermatitidis.

   d. Coccidioides immitis.

3. Name two organisms that cause histoplasmosis.

   _____

   _____

4. Histoplasmosis is a disease characterized by persistent respiratory symptoms.

   a. True.

   b. False.

5. Direct examination of a clinical specimen is diagnostic of Histoplasma, if:

    a. Branched chains of blastoconidia are seen.

    b. Brown sclerotic bodies are seen.

    c. The yeast form of the organism is seen.

    d. Blastoconidia are seen within mononuclear cells.

6. Histoplasma capsulatum can be distinguished from the contaminant Sepedonium by:

    a. Microscopic examination of microconidia.

    b. Characteristics of colonial morphology.

    c. Demonstration of diphasic nature of Histoplasma capsulatum.

    d. Origin of the clinical specimen.

7. Diagnosis of infection by Blastomyces dermatitidis is usually made by:

    a. Clinical symptoms of the disease.

    b. Recovery of the organism.

    c. A positive complement-fixation test.

    d. Presence of nodules in the lung.

8. Microconidia resembling "lollipops" are characteristic of which organisms?

    a. Histoplasma capsulatum.

    b. Sepedonium spp.

    c. Blastomyces dermatitidis.

    d. Coccidioides immitis.

9. If direct examination of a clinical specimen reveals thick-walled globose blastoconidia with buds arranged as a "pilot's wheel", the organism is likely to be:

    a. Paracoccidioides brasiliensis.

    b. Blastomyces dermatitidis.

    c. Histoplasma duboisii.

    d. None of the above.

10. Macroscopic colony morphology of Blastomyces dermatitidis and Paracoccidioides brasiliensis during their yeast phases are useful in differentiating the organisms.

    a. True.

    b. False.

11. Coccidioides immitis differs from other systemic fungi because it:

    a. Is monophasic.

    b. Has two distinct yeast phases.

    c. Is nonpathogenic.

    d. Has distinct mold and tissue phases.

12. Coccidioides immitis is spread within a host by:

    a. Release of endospores from a ruptured spherule.

    b. Ulcerated nodules in the lymphatic system.

    c. Reinfection due to improper hygiene.

    d. Transportation of organism within the RE cells.

13. Which systemic organism has barrel-shaped arthroconidia showing alternating light and dark staining cells?

_____

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 7.

1. Inhalation of viable spores. (para 7-1a)

2. a (para 7-1a)

3. Histoplasma capsulatum.
   Histoplasma duboisii. (para 7-2a)

4. b (para 7-2b)

5. d (para 7-2d(1))

6. c (para 7-2d(3))

7. b (para 7-3c(1))

8. c (para 7-3c(2))

9. a (para 7-4b)

10. b (para 7-4c(2))

11. d (para 7-5b)

12. a (para 7-5b)

13. Coccidioides immitis. (para 7-5d(2)

End of Lesson 7

LESSON ASSIGNMENT

LESSON 8	Mycological Procedures.
TEXT ASSIGNMENT	Paragraphs 8-1 through 8-12.
TASK OBJECTIVE	After completing this lesson, you should be able to:

8-1. Identify the characteristics of procedures used to process specimens for mycological studies.

8-2. Select the statement that correctly describes principles guiding application of specific methods of identifying mycological organisms.

8-3. Select the statement that describes procedures for specific methods of identifying mycological organisms.

8-4. Select the statement that describes interpretation of specific procedures for identifying mycological organisms.

SUGGESTION After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 8

MYCOLOGICAL PROCEDURES

Section I. SPECIMEN PROCESSING

8-1. INTRODUCTION

Accuracy of results obtained from mycological studies in a clinical laboratory depends on the correct application of procedures for processing specimens and identifying organisms. To apply these procedures properly, the laboratory specialist must understand the scientific principles on which those procedures are based.

8-2. KOH WITH GLYCEROL

a. **Principle**. Potassium hydroxide (KOH) serves as a clearing agent for observation of fungal elements in clinical specimens. The addition of 10 percent glycerol decreases precipitation of KOH in reagent bottles and allows the KOH preparation to be kept for 18 hours or longer.

b. **Reagents**. See Lesson 9.

c. **Procedure**.

(1) Place a drop of KOH in the center of a clean, glass, and microscope slide.

(2) Place a fragment of the tissue, purulent material, or scrapings in the KOH, and tease apart with teasing needles to produce a thin KOH preparation.

(3) Cover preparation with a cover slip, gently heat the slide by passing it through the bunsen burner flame several times.

NOTE: Use caution to ensure you do not overheat the slide.

(4) Examine the preparation using the low power (10x) objective of the microscope. Use high dry (45/47x) objective to confirm observations.

d. **Interpretation**.

(1) DO NOT REPORT AS "POSITIVE" OR "NEGATIVE"!!!

(2) If any fungal elements are seen, report as "Positive for: insert the structure/element identified. Example: "Positive for true hyphae."

(3) If no fungal elements are seen, report as "No fungal elements seen."

8-3. INDIA INK REAGENTS AND TEST

a. **Principle**. The capsule on yeast cells will repel the carbon particles in India ink results in a clear, capsular "halo" around cells.

b. **Reagents**. India ink used for this procedure is available from many commercial sources and can usually be obtained wherever art or drawing supplies are sold. For routine use, dilute the commercial item with water (2:1.) Commercially prepared ink capsules are also available.

NOTE: India ink is easily contaminated with bacteria, yeast, or fungal spores. The reagent should be checked periodically for these cells by preparing a mount, without adding specimen.

c. **Procedure**.

(1) Place two or three loopfuls (one small drop) of diluted India ink in the center of a clean glass slide.

(2) Add a drop of the specimen to be studied (body fluids, urine, and so forth). Tissue should be homogenized before using. Only a small portion should be used if working with an unknown yeast colony.

(3) Mix well. Preparation should be brownish, not black, in color. It may be diluted with sterile distilled water, if necessary, and should be covered with a cover slip.

(4) Examine the preparation using the low power (10x) objective of a microscope. Use high dry (45/47x) objective to confirm observations.

d. **Interpretation**. Presence of budding yeast cells with a clear halo is indicative of capsular material (considered a POSITIVE preparation.) If no yeast cells are observed or cells without the halo are observed, a NEGATIVE report may be submitted.

NOTE: When encapsulated yeasts are observed in a preparation of spinal fluid in India ink, Cryptococcus neoformans may be suspected. Cultural confirmation is required before the agent can be positively identified. This is because encapsulated yeasts other than C. neoformans may be seen in specimen preparations.

8-4. LACTOPHENOL COTTON BLUE STAIN

a. **Principles**. Cotton blue (China blue) stains chitin and cellulose. Since cell walls of fungi are primarily chitin, this stain is an excellent choice for observing fungi in clinical specimens.

b. **Reagents**. See Lesson 9.

c. **Procedure**.

(1) Place a small drop of lactophenol cotton blue (LPCB) in the center of a clean glass slide. Slide should be placed on a light box or sheet of white paper for easier manipulation.

(2) Remove a fragment of fungus culture with a teasing needle and place in the LPCB.

NOTE: Since a colony grows from the center outwards, it is usually advisable to take the specimen from an area 4-5 mm from the edge. This area should show characteristic structures required to identify the organism properly. The center, being the oldest section of the culture, usually shows an abundance of sterile hyphae. The periphery will not have aged sufficiently to produce any characteristic structures. If identifiable structures are not seen in this area (4-5 mm from the edge), use a fragment 8-10 mm from the outer edge and repeat the procedure. If characteristic structures are still not evident, the colony may be too young or the particular organism may not sporulate on medium. If only sterile hyphae are seen, reincubate the culture.

(3) After removing residual carbon from the teasing loops with steel wool or a towel (after flaming), gently tease apart.

(4) Gently lower a cover slip onto the preparation. Heat from the microscope lamp will spread the medium evenly.

NOTE: Do NOT press or tap the cover slip because this tends to break the conidia from the conidiophores and makes identification more difficult, or impossible.

(5) The preparation may be preserved indefinitely by sealing the edges with colored nail polish, after wiping off excess mounting medium. Colored polish lasts longer than clear polish.

(6) Examine the preparation using the low power (10x) objective of a microscope. Use a high dry (45/47x) objective to confirm observations.

d. **Interpretation**. If possible, report identification of organisms based on observed structures. If reportable structures are not seen, or structures seen are nonspecific, additional testing, such as a slide culture and/or biochemical testing may be required.

8-5. SLIDE CULTURE

a. **Principle**. Occasionally, conidia from a culture may not be observed in a Lactophenol Cotton Blue preparation. By growing the organism in a way that allows periodic observation, the precise time that conidia are at prime identifiable stage can be determined.

b. **Equipment**.

(1) Sterile petri dish (15 x 100 mm).

(2) Filter paper (100 mm diameter).

(3) Sterile, distilled water.

(4) Sterile pipettes (graduated or Pasteur).

(5) Sterile, rimless test tube (approx. 15 mm in diameter.) This is an optional item.

(6) Sterile scalpel.

(7) Petri dish with potato dextrose agar (PDA) or other medium of choice. Plate should contain 15 ml of agar.

(8) Sterile microscope slides and cover slips.

c. **Procedure**.

(1) Flame rim of sterile test tube and allow to cool. Use tube or scalpel to cut plugs or squares in agar plate.

(2) With flat side of scalpel, transfer agar plug to center of sterile slide. Set slide atop a piece of filter paper placed in the bottom of a sterile petri dish.

(3) With a sterile probe, inoculate the edges of the agar in 3 or 4 areas with small fragments of the fungal colony.

(4) Use sterile forceps to place a sterile coverslip on top of the agar plug.

(5) Moisten the filter paper by placing approximately 1.5 ml of sterile distilled water along the edge of the petri dish, allowing the filter paper to absorb it. Do not allow water to get on surface of slide.

(6) Incubate culture as required (usually room temperature.)

d. **Interpretation**.

(1) Examine culture every other day. Remove slide from petri dish and wipe off any condensation on bottom of slide. Place slide on stage of microscope and observe using low power (10x) and/or high dry (45/47x). Examine for development of identifiable structures.

(2) If culture has not reached the proper growth stage yet, replace culture in moist chamber, and reincubate. Additional water may be added when necessary. However, if too much water is added, culture may become contaminated.

(3) If slide culture has developed identifiable structures, gently lift cover slip off agar surface with pair of sterile forceps, and place on slide containing drop of LPCB. If agar block sticks to coverslip, gently remove with a sterile scalpel or probe. Preparation may be preserved indefinitely by sealing edges with nail polish, after removing excess mounting media.

(4) Examine preparation using low power (10x) objective of a microscope. Use high dry (45/47x) objective to confirm observations.

(5) If possible, report organism identification based on observed structures. If reportable structures are not seen or structures seen are nonspecific, additional testing such as repeat slide culture and/or biochemical testing may be required.

Section II. ORGANISM IDENTIFICATION

8-6. GERM TUBE TEST

a. **Principle**. This procedure is used to differentiate Candida albicans and Candida stellatoidea from other yeasts.

b. **Reagents**. See Lesson 9, paragraph 9-9.

c. **Procedure**.

(1) Emulsify a small amount of yeast culture in a tube of germ tube medium.

(2) Incubate the tube for 1 hour at 35º C.

(3) Examine the culture by placing a loopful of the serum culture on a clean glass slide. Coverslip and observe microscopically for germ tube formation.

(4) If germ tubes are not seen, reincubate as long as an additional 2 hours and repeat step (3) every 15 minutes.

NOTE: The test is examined within 3 hours because yeasts develop pseudohyphae after incubating three hours.

d. **Interpretation**.

(1) Positive: Formation of germ tubes.

(2) Negative: Nonformation of germ tubes.

e. **Quality Control**.

(1) Positive: Candida albicans.

(2) Negative: Candida tropicalis.

8-7. STERILE RICE GRAIN TEST

a. **Principle**. This procedure is used for differentiation of Microsporum spp, H. canis, H. gypseum, and sporulation of other dermatophytes. H. audouinii produces only negligible growth.

b. **Reagents**. See Lesson 9.

c. **Procedure**.

(1) Inoculate a flask of rice grain medium with several fragments of fungal culture. Be careful not to transfer any portion of the SDA medium.

(2) Incubate at room temperature for 1-2 weeks.

(3) Examine for growth on a periodic basis.

(4) Optional: When growth is sufficient, prepare a tease mount and examine for the presence of spores.

d. **Interpretation**.

(1) Positive: growth.

(2) Negative: no growth.

e. **Quality Control**.

(1) Positive: Microsporum canis.

(2) Negative: Microsporum audouinii.

8-8. UREASE TEST

a. **Principle**. This test determines the ability of an organism to hydrolyze urea.

b. **Reagents**. Christensen's urea agar. Prepare according to manufacturer's instructions.

c. **Procedure**.

(1) Inoculate two tubes of urea agar with fragments of mold or yeast culture being tested.

(2) Incubate one tube at 25º C and the other at 35º C.

(3) Observe daily for one week for change in color to red.

d. **Interpretation**.

(1) Positive: pink to red color developed within 7 days.

(2) Negative: no pink to red color developed or color developed after 7 days.

e. **Quality Control for Molds**.

(1) Positive: Trichophyton mentagrophytes var. mentagrophytes.

(2) Negative: Trichophyton rubrum.

f. **Quality Control for Yeasts**.

(1) Positive: Cryptococcus albidus.

(2) Negative: Candida albicans.

8-9. IN VITRO HAIR TEST

a. **Principle.** This test is used to differentiate Trichophyton mentagrophytes and Trichophyton rubrum.

b. **Reagents**.

(1) Sterile, distilled water.

(2) Sterile petri dishes (glass or plastic).

(3) Sterile glass bottle (if glass petri dishes are not available).

(4) Sterile forceps.

(5) CLEAN human hair (ensure it has not been sprayed.)

(6) Ten percent sterile yeast extract (prepared fresh monthly.) Filter, sterilize.

c. **Procedure**.

(1) Put short strands of CLEAN human hair in glass petri dish or bottle.

NOTE: Best results are obtained if hair is from a pre-adolescent child.

(2) Sterilize in an autoclave for 15 minutes at 121º C.

(3) If hair is sterilized in a glass bottle, transfer aseptically to plastic petri dish with sterile forceps.

(4) Add 25 ml of sterile, distilled water.

(5) Add 0.1 ml (2 or 3 drops) of sterile yeast extract, 10 percent.

(6) Add several fragments of fungal culture being tested.

(7) Incubate at room temperature.

(8) Examine at 7 and 14 days.

(a) Remove several hair strands with sterile forceps.

(b) Mount in LPCB and examine. Gentle heating will aid stain penetration of the mycelium.

d. **Interpretation**.

(1) Positive: wedge shaped perforations of the hair.

(2) Negative: no perforation of hair during the 28-day test.

NOTE: Perforations usually are not abundant until the10 to 14th day of test.

e. **Quality Control**.

(1) Positive: Trichophyton mentagrophytes.

(2) Negative: Trichophyton rubrum.

8-10 PERIODIC ACID-SCHIFF (PAS) STAIN

a. **Principle**. The periodic acid-Schiff procedure is used to stain fungi as well as tissue. Stain reacts with polysaccharides found in cell walls of fungi. Periodic acid, acting as an oxidizing agent, breaks the C-C bonds at the 1:2 glycol sites within polysaccharides. Hydroxyl groups are converted to aldehyde radicals. Basic fuchsin combines with the aldehyde groups and forms a bond that is strong enough to withstand the bleaching effect of sodium metabisulfite.

b. **Reagents**.

(1) See Lesson 9 for PAS.

(2) Spread into thin smear Ethanol solutions; aqueous, 70 percent, 85 percent, and 95 percent.

(3) Absolute ethanol.

(4) Xylene.

(5) Mounting medium.

(6) Sterile, wooden applicator sticks.

(7) Microscope slides with frosted ends.

(8) Coplin jars or other staining dishes.

(9) Slide warmer.

(10) Incubator, 35º C.

(11) Microscope.

(12) Cover slips.

(13) Control slides prepared from tissue or sputum known to contain yeast.

c. **Procedure**.

(1) Preparation of smears from clinical specimens.

(a) Skin and nail scrapings.

1 Place a thin film of albumin on a clean, glass slide.

2 Embed several thin skin or nail scrapings in the film by placing them on top of the albumin and gently pressing down with another clean, glass slide.

3 Allow smear to air dry for several hours. Drying may be speeded up by placing the slide on a slide warmer or in an incubator.

NOTE: DO NOT HEAT FIX OVER A FLAME!

(b) Tissue.

1 Prepare a homogenate from the tissue.

2 Place a drop in the center of a clean glass slide.

3 Spread into a thin smear.

4 Allow to air dry.

(c) Exudates/purulent material.

1 Place a drop of water the center of a center glass slide.

2 Emulsify a loopful of material in the water and spread across the slide.

3 Allow to air dry.

(2) Staining procedure.

(a) Place fungal smear in absolute ethanol for 1 minute.

(b) Drain alcohol, and immediately place slide in 5 percent periodic acid solution for 5 minutes.

(c) Wash in running water for 2 minutes.

(d) Place in basic fuchsin solution for 2 minutes.

(e) Wash in running water for 2 minutes.

(f) Place in sodium metabisulfite for 5 minutes.

(g) If employing counterstain, see technique listed below in (3).

(h) If counterstain is not used, dehydrate by passing through 70 percent, 85 percent, 95 percent, and absolute alcohol for 2 minute intervals.

(i) Place in xylene for 2 minutes and mount with cover slip and Permount. Do not allow slide to dry before mounting cover slip.

(3) Technique for staining employing counterstain.

(a) Following removal of slide from metabisulfite, wash 5 minutes in running water.

(b) Place slide in light green stain for 5 seconds.

(c) Wash for 5-10 seconds.

d) Dip slide for 5 second intervals in 85 percent, 95 percent, and absolute alcohols.

(e) Dip in xylene and mount with Permount.

d. **Interpretation**.

(1) Observe control slides first. Fungal elements stain a magenta color and background appears pink to red, depending on thickness of preparation. Potential problems areas include:

(a) Periodic acid may deteriorate, resulting in a lack of staining of fungal elements. Periodic acid solution should be kept in a dark bottle.

(b) Sodium metabisulfite may deteriorate, resulting in lack of bleaching, causing the background to be as darkly stained as fungal elements.

(c) If light green counterstain is used, background will appear to be green. Fungal elements will appear to be magenta.

(2) Report results of the control slide using criteria outlined above, interpret the test slide.

NOTE: Bacteria and neutrophils may retain basic fuchsin along with fungal elements. This should not cause any interpretation difficulties.

8-11. SALINE WET MOUNT

a. **Principle**. Saline mounts are used to examine clinical specimens and pure cultures of yeasts.

b. **Reagents and Equipment**.

(1) Sterile saline.

(2) Sterile, glass microscope slides.

(3) Sterile cover slips.

(4) Microscope.

c. **Procedure**.

(1) Place a small drop of saline on a clean, glass slide.

(2) Suspend a small amount of clinical specimen or culture in the saline.

(3) Gently cover slip the preparation.

d. **Interpretation**. Examine microscopically for presence of ascospores, as well as size, shape, and other characteristics of blastoconidia. Describe the fungal elements observed.

8-12. CUT-STREAK METHOD FOR MORPHOLOGY OF YEASTS

a. **Principle**. Many yeasts procedures show similar biochemical test results, making differentiation difficult. These same yeasts may have very distinctive microscopic morphologies. By inoculating them to a "starvation medium" such as cornmeal agar, sporulation may be induced, which will allow observation of these characteristic morphologies.

b. **Reagents and Equipment**.

(1) See Lesson 9 for preparation of starvation medium.

(2) Inoculating needles.

(3) Sterile slides and cover slips.

(4) Forceps.

c. **Procedure**.

(1) Touch an inoculating needle to a 24 to 72 hour old yeast culture.

(2) Make two, parallel scratches, several inches long, just below the surface of the agar.

CAUTION: DO NOT CUT THE AGAR DEEPLY!

(3) Make several zigzag streaks across the original scratches.

(4) Place a sterile coverslip over a portion of the scratches.

(5) Incubate at room temperature for 18 to 24 hours. Some isolates may require incubation for as long as 72 hours.

(6) Remove lid of petri dish and observe microscopically (10x or 45/47x).

d. **Interpretation**. Examine for characteristic microscopic morphologies of yeasts. Report structures observed, such as arthroconidia, blastoconidia, pseudohyphae, chlamydospores, and so forth. It may also prove helpful to observe macroscopic colony morphology.

Continue with Exercises

EXERCISES, LESSON 8

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Potassium hydroxide (KOH) with glycerol acts as:

   a. A preservative.

   b. A clearing agent.

   c. A stain.

   d. An antifungal agent.

2. A positive India ink test is indicative of the presence of:

   a. Encapsulated yeast.

   b. Pathogenic mold.

   c. Nonencapsulated yeast.

   d. Pseudohyphae.

3. Lactophenol cotton blue is useful for microscopic examination of fungal cultures because it:

   a. Acts as a clearing agent.

   b. Confirms results of the India ink test.

   c. Stains walls of the fungi.

   d. All of the above.

4. The reason for using a slide culture procedure is _____

_____

_____

5. Which of the following represents the usual temperature use to incubate a slide culture?

   a. 0º C.

   b. 30º C.

   c. 37º C.

   d. Room temperature.

6. A positive germ tube test indicates:

   a. Any Candida spp.

   b. Candida albicans.

   c. Candida tropicalis.

   d. Microsporum audouinii.

7. Which phase of fungal growth is studied in the Urease test?

   a. Mold phase.

   b. Yeast phase.

   c. Both mold and yeast phases.

   d. None of the above.

8. Which color develops as a result of a positive urease test?

    a. Red.

    b. White.

    c. Yellow.

    d. Black.

9. The "in vitro" hair test requires the sterilization of several strands of clean hair.

    a. True.

    b. False.

10. An "in vitro" hair test is observed at:

    a. 5 and 10 days.

    b. 10 and 14 days.

    c. 16 days.

    d. 28 days.

11. What organism produces a positive sterile rice grain test?

    a. Microsporum canis.

    b. Candida albicans.

    c. Microsporum audouinii.

    d. Trichophyton rubrum.

12. In the periodic acid-Schiff stain procedure, sodium metabisulfite functions as an:

    a. Oxidizing agent.

    b. Bleaching agent.

    c. Staining agent.

    d. Dehydrating agent.

13. The fuchsin stain on slides processed with the Periodic Acid-Schiff Stain procedure is retained by:

    a. Fungi cell walls.

    b. Bacteria.

    c. Neutrophils.

    d. All of the above.

14. What is the category of culture media used for the Cut-Streak procedure?

    a. Nutrient media.

    b. Isolation media.

    c. Starvation media.

    d. Enhanced media.

15. The cut-streak procedure induces _____, thereby allowing for microscopic observation for characteristic _____.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 8

1. b. (para 8-2a)

2. a. (para 8-3d)

3. c. (para 8-4a)

4. Growing the organism in a way that allows periodic observation, the precise time that conidia are at prime identifiable stage can be determined. (para 8-5a)

5. d. (para 8-5c(6))

6. d. (para 8-6e)

7. c. (para 8-8c(1))

8. c. (para 8-8d)

9. a. (para 8-9c(2))

10. b. (para 8-9d(8))

11. a. (para 8-7e(1))

12. b. (para 8-10a)

13. d. (para 8-10d(NOTE)

14. c. (para 8-12a)

15. Sporulation
Morphologies. (para 8-12a)

End of Lesson 8

LESSON ASSIGNMENT

LESSON 9	Media and Reagents.
TEXT ASSIGNMENT	Paragraphs 9-1 through 9-13.
TASK OBJECTIVES	After completing this lesson, you should be able to:
	9-1. Select the statement that correctly describes characteristics of specific mycologic isolation media.
	9-2. Select the statement that correctly describes characteristics of specific mycologic reagents.
SUGGESTION	After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 9

MEDIA AND REAGENTS

Section I. MEDIA

9-1. INTRODUCTION

To perform effective diagnostic mycology, the media and reagents used for culture of organisms must be accurately prepared. Preparation requires an understanding of the principles of mycological media and reagents.

9-2. ISOLATION MEDIA

Isolation media are used to recover and isolate fungi from clinical specimens.

9-3. SABOURAUD'S DEXTROSE AGAR

a. **Contents**. Sabouraud dextrose agar (SDA) consist of the following.

Neopeptone	10.0 gm
Glucose	20.0 gm
Agar	15.0 gm
Distilled water	1.0 liter

b. **Preparation**.

(1) Mix ingredients.

(2) Bring to a boil. Adjust pH to 5.5-6.0.

(3) Dispense 7.0 mL aliquots into 16 x 125 mm screw capped test tubes.

(4) Autoclave for 10 minutes at 15 psi.

(5) Allow tubed media to solidify in a slanted position.

(6) Storage: 4º C.

(7) Shelf life: 30 days in test tubes, 14 days in Petri dishes.

c. **Quality Control**.

(1) Sterility.

(2) Color.

(3) Performance. See below.

Fungus	Incubation	Results
Aspergillus flavus	30º C.	Good growth

NOTE: Sabouraud dextrose agar can also be dispensed in 15.0 mL aliquots into sterile Petri dishes. Glucose concentration can be increased to 40.0 gm per liter. Medium is recommended for studying colonial morphology of dermatophytes. SDA is commercially available and should be prepared according to manufacturer's instructions. pH 5.5-6.0 inhibits growth of bacterial contaminants and enhances pigmentation of dermatophytes. pH 7.0 is excellent for isolation of systemic organisms.

9-4. SABOURAUD DEXTROSE AGAR WITH ANTIBIOTICS (MYCOSEL™)

a. **Contents**. Sabouraud dextrose agar with antibiotics consists of the following.

Peptone	10.0 g.
Glucose	10.0 g.
Cycloheximide	0.5 g.
Chloramphenicol	0.05 g.
Agar	15.0 g.
Distilled water	1.0 liter

b. **Preparation**.

(1) Dissolve cycloheximide in 10.0 of acetone.

(2) Dissolve chloramphenicol in 10.0 mL of 95 percent ethanol.

(3) Mix ingredients cycloheximide, and chloramphenicol.

(4) Bring to a boil. Adjust pH to 6.8 - 7.0.

(5) Dispense in 7.0 mL aliquots in 16 x 125 mm screw capped test tubes.

(6) Autoclave for 10 minutes at 15 psi.

(7) Slant test tubes.

(8) Storage: 4º C.

(9) Shelf life: 30 days in test tubes, 14 days in Petri dishes.

c. **Quality Control.**

(1) Sterility.

(2) Color.

(3) Performance. See below.

Fungus	Incubation	Results
Trichophyton mentagrophytes	30º C.	Good growth
Aspergillus elavus	30º C.	No Growth

NOTE: Mycosel™ and Mycobiotic™ Agars are two commercially prepared media. They should be prepared according to the manufacturer's instructions. Sabouraud dextrose agar with chloramphenicol is prepared as above except cycloheximide is omitted. Media with antibiotics are useful for isolation of fungi from contaminated sites.

9-5. BRAIN HEART INFUSION AGAR

a. **Contents.** Brain heart infusion agar (BHIA) ingredients are as follows.

Calf brains, infusion	200.0 g.
Beef heart, infusion	250.0 g.
Peptone	10.0 g.
Glucose	2.0 g.
Sodium chloride	5.0 g.
Disodium phosphate	2.5 g.
Agar	15.0 g.
Distilled water	1.0 liter

b. **Preparation.**

(1) Mix ingredients in a 2-liter flask.

(2) Bring to a boil. Adjust pH to 7.4.

(3) Dispense 7.0 mL aliquots in 16 x 125 mm screw capped test tubes or 15.0 mL aliquots into 25 x 125 mm screw capped test tubes.

(4) Autoclave for 15 minutes at 15 psi.

(5) Slant test tubes.

(6) Storage: 4º C.

(7) Shelf life: 30 days in test tubes, 14 days in Petri dishes.

c. **Quality Control**.

(1) Sterility.

(2) Performance. See below.

Fungus	Incubation	Results
Aspergillus flavus	30º C.	Good growth

NOTE: Commercially available media, prepared according to manufacturer's instructions, may be substituted. Brain heart infusion agar: in test tubes can be melted and poured into sterile Petri dishes. It can be enriched with 5 percent sheep blood. It supports growth of fastidious organisms, and can be used to convert diphasic organisms to yeast forms.

9-6. POTATO DEXTROSE AGAR

a. **Contents**. The contents of potato dextrose agar (PDA) are as follows.

Potato infusion	500.0 mL
Glucose	10.0 g.
Agar	15.0 g.
Tap water	1.0 liter

b. **Preparation**.

(1) Peel and dice 200.0 g. of old-crop potatoes.

(2) Add potatoes to 500.0 mL of tap water.

(3) Boil for one hour or cook in autoclave for 10 minutes at 15 psi.

(4) Filter through cheesecloth.

(5) Q.S. to 500.0 mL using tap water.

(6) Add ingredients to the infusion.

(7) Bring to a boil.

(8) Dispense 7.0 mL aliquots into 16 x 125 mm screw capped test tubes.

(9) Autoclave for 15 minutes at 15 psi.

(10) Slant test tubes.

(11) Storage: 4º C.

(12) Shelf life: 30 days in test tubes. 14 days in Petri dishes.

c. **Quality Control**.

(1) Sterility.

(2) Performance. See below.

Fungus	Incubation	Results
Aspergillus flavus	30º C.	Good growth

NOTE: Potato dextrose agar (PDA) is commercially available. It enhances sporulation and pigment production.

9-7. CORNMEAL AGAR

a. **Contents.** Cornmeal agar (CMA) consists of the following.

Cornmeal	50.0 g.
Agar	15.0 g.
Distilled Water	1.0 liter

b. **Preparation**.

(1) Mix cornmeal in 500.0 mL of distilled water.

(2) Boil for one hour, or autoclave for 10 minutes at 15 psi.

(3) Filter suspension through cheesecloth.

(4) Q.S. to one liter using distilled water, and add agar.

(5) Bring to a boil.

(6) Dispense 7.0 mL aliquots into 16 x 125 mm screw capped test tubes.

(7) Autoclave for 15 minutes at 15 psi.

(8) Slant test tubes.

(9) Storage: 4º C.

(10) Shelf life: 30 days in test tubes. 14 days in Petri dishes.

c. **Quality Control**.

(1) Sterility.

(2) Performance. See below

Organism	Incubation	Results
Aspergillus flavus	30º C.	Good growth and conidia production
Trichophyton rubrum	30º C.	Red pigment
Candida albicans	30º C.	Chlamydospores

NOTE: Selection of quality control organism should be based on expected use of the medium.

NOTE: Commercially prepared media are also available. If cornmeal agar is used for chlamydospore development in the Dalmau technique, 10.0 mL of Tween 80 is added to the medium prior to sterilization. After sterilization, dispense in 15.0 mL aliquots into sterile Petri dishes. This is a starvation medium that causes chlamydospore production by some yeasts.

9-8. CREAM OF RICE AGAR WITH TWEEN 80

a. **Contents**. Cream of rice agar with Tween 80 consists of the following.

Cream of rice	10.0 g.
Agar	10.0 g.
Tween 80	10.0 mL
Distilled water	1.0 liter

b. **Preparation.**

(1) Boil rice in water 1 minute; simmer for 3 minutes.

(2) Filter through several layers of gauze.

(3) Add agar and Tween 80 to filtrate.

(4) Q. S. to 1 liter using distilled water.

(5) Bring to boil.

(6) Autoclave for 15 minutes at 15 psi.

(7) Dispense 15 mL aliquots into Petri dishes.

(8) Storage: 4º C.

(9) Shelf life: 30 days in test tubes. 14 days in Petri dishes.

c. **Quality Control.**

(1) Sterility.

(2) Performance. See below.

Organism	Incubation	Results
Candida albicans	30º C.	Chlamydospores

NOTE: Commercially prepared media is also available.

9-9. GERM TUBE MEDIA

a. **Contents**. Germ tube media consists of the following.

Pooled human serum Horse serum. Fetal calf serum. Bovine serum.

b. **Preparation**.

(1) Dispense 0.3 mL aliquots into 12 x 75 mm test tubes.

(2) Cap.

(3) Storage: 70º C.

(4) Shelf life: one year.

c. **Quality Control**. Incubate at 37º C. for one hour. Observe microscopically for germ tube formation. If negative, reincubate as much as an additional two hours, observing at 15 minute intervals.

Organism	Results
Candida albicans	Germ tubes present
Candida tropicalis	Germ tubes absent

NOTE: Bovine serum may be substituted. Each lot of human serum must be evaluated before it is used. Incubation times should also be checked for each lot of medium prepared.

9-10. RICE GRAINS

a. **Contents**. Rice grain consists of the following.

Unfortified white rice grains	8.0 g.
Distilled water	25.0 mL

b. **Preparation.**

(1) Mix ingredients in 125 mL flask.

(2) Autoclave for 15 minutes at 15 psi.

(3) Storage: 4º C.

(4) Shelf life: 30 days.

c. **Quality Contro**l.

(1) Sterility.

(2) Performance--not necessary prior to use.

NOTE: Differentiation of specific dermatophytes is based on their ability to develop on rice grains.

Section II. REAGENTS

9-11. LACTOPHENOL COTTON BLUE MOUNTING MEDIA

a. **Contents**. Lactophenol cotton blue mounting media consists of the following.

Phenol, concentrated	20.0 g.
Lactic acid	20.0 mL
Glycerol	40.0 mL
Cotton blue	0.05 g.
Distilled water	20.0 mL

b. **Preparation**.

(1) Dissolve cotton blue in distilled water.

(2) Add phenol, lactic acid and glycerol.

NOTE: Melt phenol before weighing.

(3) Storage: room temperature.

(4) Shelf life: one year (filter if dye precipitates out of solution).

c. **Quality Control**. Not required.

NOTE: Lactic acid assists preservation of fungal structures. Phenol functions as a killing agent. Cotton blue functions as a chitin stain.

9-12. POTASSIUM HYDROXIDE SOLUTIONS

a. **Contents**.

(1) Ten percent potassium hydroxide solutions (KOH). See below.

Potassium hydroxide	10.0 g.
Distilled water	100.0 mL

(2). Ten percent potassium hydroxide solutions with glycerol. See below

Potassium hydroxide	10.0 g.
Glycerol	20.0 g.
Distilled water	100.0 mL

b. **Preparation**.

(1) Mix ingredients in order listed.

(2) Storage: room temperatures.

(3) Shelf life: six months.

c. **Quality Control**. Not required.

NOTE: Acts as clearing agent for observation of fungal elements in clinical specimens. Glycerol retards precipitation of KOH crystals.

9-13. PERIODIC ACID-SCHIFF STAIN REAGENTS

a. **Contents**. Periodic acid schiff stain reagents (PAS) contain the following.

(1) Periodic acid solution. See below.

Periodic acid	5.0 g.
Distilled water	100.0 mL

(2) Basic fuchsin solution. See below.

Basic fuchsin	0.1 g.
Ethanol, absolute	5.0 mL
Distilled water	95.0 mL

(3) Sodium metabisulfite solution. See below.

Sodium metabisulfite	1.0 g.
Hydrochloric acid, IN	10.0 mL
Distilled water	190.0 mL

(4) Formula for counterstain. See below.

Light Green crystals	0.2 g.
Glacial acetic acid	2.0 g.
Distilled water	100.0 mL

(5). Ethanol solutions. Ethanol solutions are aqueous, 70 percent, 85 percent, and 95 percent Ethyl Alcohol and Absolute Ethyl Alcohol.

(6) Xylene. Mounting medium.

b. **Preparation**. Prepare all solution in brown, light resistant, bottles.

(1) Periodic acid solution. Mix ingredients, tighten bottle cap.

(2) Basic fuchsin. Mix ethanol and water in bottle, add basic fuchsin, mix gently, and tighten bottle cap.

(3) Sodium metabisulfite. Add HCL to water in bottle, add sodium metabisulfite, and tighten bottle cap.

(4) Storage: 4º C., in brown bottle.

(5) Shelf life: several days. Discard when controls no longer stain properly.

c. **Quality Control.**

(1) Keep stock period acid powder in a desiccator.

(2) Permanent stain for fungal elements.

Continue with Exercises

EXERCISES, LESSON 9

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Sabouraud dextrose agar (SDA) is recommended for:

   a. Studying the colonial morphology of dermatophytes.

   b. Culturing specimens form contaminated sites.

   c. Growing diphasic organisms.

   d. Growing fastidious organisms.

2. Sabouraud dextrose agar media with a pH of 5.7 will enhance:

   _____

3. The use of SDA with antibiotics is indicated when:

   a. Systemic organisms are suspected.

   b. The source of the culture material is contaminated.

   c. The pH of culture media must be controlled.

   d. The suspected fungus is a rapid grower.

4. The incubation temperature for brain-heart infusion agar (BHIA) is:

   a. 4º C.

   b. 27º C.

   c. 30º C.

   d. 37º C.

5. During preparation of potato dextrose agar (PDA), the infusion is:

   a. Filtered after each boiling.

   b. Filtered after initial boiling.

   c. Filtered after addition of glucose.

   d. Not filtered.

6. When grown on cornmeal agar, Trichophyton rub rum produces:

   a. White colonies.

   b. Chlamydospores.

   c. A red pigment.

   d. Very little growth.

7. Starvation media is used to:

   a. Support a fastidious organism.

   b. Enhance pigmentation.

   c. Convert diphasic organisms.

   d. Cause formation of chalmydospores.

8. At what temperature should germ tube media be stored? _____:

9. Cream-of-Rice agar with Tween 80 is an isolation media.

   a. True.

   b. False.

10. Lactophenol cotton blue mounting media:

   a. Preserves structures.

   b. Stains structures.

   c. All of the Above.

   d. None of the Above.

11. The amount of glycerol needed to prepare 100 mL of 10 percent KOH with glycerol is:

   a. 1 mL.

   b. 10 mL.

   c. 20 mL.

   d. 100 mL.

12. What is the shelf life of Periodic Acid-Schiff Stain?

   a. Several hours.

   b. Several days.

   c. Several weeks.

   d. Indefinitely.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 9

1. a (para 9-3)
2. Pigmentation of dermatophytes. (para 9-3)
3. b (para 9-4)
4. c (para 9-5)
5. b (para 9-6)
6. c (para 9-7)
7. d (paras 9-7, 9-8)
8. -70º C. (para 9-9)
9. b (para 9-8)
10. d (para 9-11)
11. c (para 9-12)
12. b (para 9-13)

End of Lesson 9

LESSON ASSIGNMENT

LESSON 10	Maintaining Stock Cultures.
TEXT ASSIGNMENT	Paragraphs 10-1 through 10-6.
TASK OBJECTIVES	After completing this lesson, you should be able to:
	10-1. Identify principles for maintaining a "working" stock culture.
	10-2. Identify procedures used to recover organisms from a stock culture.
SUGGESTION:	After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 10

MAINTAINING STOCK CULTURES

10-1. INTRODUCTION

a. Several reasons for maintaining a fungal stock culture collection exist. The most important is the need to maintain quality control of media. A stock culture collection is a valuable source of reference cultures for the medical technologist who must identify atypical or unfamiliar fungal isolates on a daily basis. The ability to refer to known cultures and compare their properties with those of unknown isolates is often of great advantage. Finally, laboratories where new technicians are trained in diagnostic medical mycology must have a stock culture collection that represents, at the very least, those fungi most frequently isolated from clinical specimens.

b. However, keeping a set of stock cultures on the shelf is insufficient. Keeping those cultures sporulating and demonstrating characteristic colonial morphologies is required to maintain a collection. Showing a new technician a white, woolly, non-sporulating culture, the back of which is light brown, and expecting the technician to recognize it as an isolate of Trichophyton rubrum is unsatisfactory. Two major procedures are required to maintain a stock fungal culture collection properly. First is to keep the organism viable until it is needed. Second is to retain typical colonial and microscopic morphologies of each fungus.

10-2. MAINTENANCE OF "WORKING" STOCK CULTURES

a. Some mycology laboratories who have continuous training programs must maintain stock cultures ready for use on short notice. These cultures are called "working" stocks.

b. "Working" stock cultures must be subcultured at regular intervals (four to six weeks), checked for presence of spores and development of sterile, pleomorphic mycelium. When pleomorphism develops, great effort must be made to avoid overgrowth of the typical mycelium by pleomorphic portions, or the entire culture will be lost.

c. Several media may be required to maintain a culture that is typical and sporulating. Some fungi require alternating between two or three media to maintain their characteristic gross and microscopic morphologies.

d. The following table summarizes media used to maintain "working" stocks in the Mycology Training Branch at Center Disease Control. It should be noted that each stock culture is checked frequently for sporulation (table 10-1).

Maintenance of Working Stock Fungus Cultures

Fungus	Media (Listed at bottom of page)
Microsporum audouinii	Sab. (1)
Microsporum canis	d.s. (2); every third subculture should include Sab.
Microsporum gypseum	Rotate between Pd (3) and d.s.
Microsporum cookei	Sab.; if becoming pleomorphic, rotate between Sab. and d.s.
Microsporum persicolor	Sab.; may occasionally rotate with G-7 (6) or BL-S (4)
Microsporum nanum	Sab.; may occasionally rotate with d.s.
Microsporum distortum	Rotate between Sab. and BL-S, and medium # 1 (5)
Microsporum ferrugineum	Sab.
Trichophyton mentagrophytes var. *interdigitale*	Rotate between Sab., BL-S, and medium # 1
Trichophyton mentagrophytes var. *mentagrophytes*	Keep on d.s. for spores; rotate between d.s., Pd or Sab. or G-5.5 (7)
Trichophyton rubrum	Rotate between Pd and medium # 1; enhancement of pigment on G-7

1. Sab. - Sabouraud dextrose agar with neopeptone, pH 5.5 - 6.0, Difco
2. d.s. - Dilute Sabouraud dextrose agar
3. Pd - Henrici's potato dextrose agar
4. BL-S - Sabouraud dextrose agar, polypeptone, pH 7.0, Bioquest
5. Medium #1 - Trichophyton medium #1, Difco
6. G-7 - Mycobiologic agar, pH 7.0, Gibco
7. G 5.5 - Sabouraud agar, pH 5.5, Gibco
8. S+T - Sabouraud agar, pH 5.5, Difco + Thiamine
9. BHI - Brain Heart Infusion agar

Table 10-1. Maintenance of "working" stock fungus cultures (continued).

Fungus	Media (Listed at bottom of page)
Trichophyton tonsurans	Sab., rotate occasionally with S+T (8)
Trichophyton terrestre	Sab. or Pd; check sporulation
Trichophyton ajelloi	BL-S and Sab.; d.s. to maintain sporulation
Trichophyton verrucosum	S+T
Trichophyton schoenleinii	Sab.
Trichophyton concentricum	Sab.
Trichophyton violaceum	Rotate between Sab. and S+T (Sab --> S+T; S+T -->Sab.); both media used at all times
Trichophyton megninii	Sab.; BL-S occ. for better pigment
Trichophyton soudanense	Sab.
Trichophyton gallinae	Rotate between Sab. and S+T (see *Trichophyton violaceum*)
Epidermophyton floccosum	Sab.; occasionally rotating with BL-S may enhance sporulation but causes gross morphology changes; culture may die if kept on BL-S
Cladosporium spp.	Pd; occasionally rotate with 18% V-8 agar to enhance sporulation
Fonsecaea pedrosoi	Same as *Cladosporium*
Phialophora verrucosa	Same as *Cladosporium*
Sporothrix schenckii	Pd

1. Sab. - Sabouraud dextrose agar with neopeptone, pH 5.5 - 6.0, Difco
2. d.s. - Dilute Sabouraud dextrose agar
3. Pd - Henrici's potato dextrose agar
4. BL-S - Sabouraud dextrose agar, polypeptone, pH 7.0, Bioquest
5. Medium #1 - Trichophyton medium #1, Difco
6. G-7 - Mycobiologic agar, pH 7.0, Gibco
7. G 5.5 - Sabouraud agar, pH 5.5, Gibco
8. S+T - Sabouraud agar, pH 5.5, Difco + Thiamine
9. BHI - Brain Heart Infusion agar

Table 10-1. Maintenance of "working" stock fungus cultures (continued).

Fungus	Media (Listed at bottom of page)
Histoplasma capsulatum	Pine's mycelium medium and yeast-form medium
Blastomyces dermatitidis	Sab. for mycelium; BHI (9) for yeast
Paracoccidioides brasiliensis	Same as for *B. dermatitidis*
Coccidioides immitis	Sab.
Candida, *Cryptococcus*, *Torulopsis* and *Trichosporon* spp.	Yeast malt extract agar; Sab. is satisfactory
Geotrichum spp.	Sab. or Pd
Aspergillus spp.	Pd
Penicillium spp.	Pd
Fusarium spp.	Sab. for color; Pd for sporulation
Chrysosporium spp.	Pd; rotate occasionally with Sab.
Sepedonium spp.	Rotate between Sab. and Pd
Scopulariopsis spp.	Pd
Nocardia spp.	7H10 or 7H11
Actinomadura spp.	7H10 or 7H11
Streptomyces spp.	Rotate between 7H10 , Sab, and BHI; isolates vary with preferences for these media

1. Sab. - Sabouraud dextrose agar with neopeptone, pH 5.5 - 6.0, Difco
2. d.s. - Dilute Sabouraud dextrose agar
3. Pd - Henrici's potato dextrose agar
4. BL-S - Sabouraud dextrose agar, polypeptone, pH 7.0, Bioquest
5. Medium #1 - Trichophyton medium #1, Difco
6. G-7 - Mycobiologic agar, pH 7.0, Gibco
7. G 5.5 - Sabouraud agar, pH 5.5, Gibco
8. S+T - Sabouraud agar, pH 5.5, Difco + Thiamine
9. BHI - Brain Heart Infusion agar

Table 10-1. Maintenance of "working" stock fungus cultures (concluded).

10-3. PLACING STOCK CULTURES IN STERILE DISTILLED WATER

An excellent method of maintaining stock cultures is to place fungal cultures in sterile, distilled water. Two groups of organisms that can easily be entered into water stocks and recovered when needed are yeasts and aerobic actinomycetes. Entering mold cultures into water requires dexterity on the part of the technologist.

a. **Placing Yeast Cultures into Water**.

(1) Inoculate yeast to a Sabouraud or yeast malt extract agar slant. Incubate at room temperature for 48 to 96 hours.

(2) Add approximately 4 mL of sterile, distilled water to the slant.

(3) Gently agitate the slant on a mechanical mixer. When most of the growth on the slant has gone into suspension, draw off aqueous suspension and place in a small, sterile, screw-capped vial or small, screw-capped test tube.

(4) Ring cap with parafilm and store container in a dust-free area at room temperature or at 20º C. Most stocks remain viable for four or five years.

b. **Placing Actinomycetes and Mold Cultures in Water**.

(1) Inoculate one or two tubes of 7H1O, potato dextrose or Sabouraud agar, depending upon the organism. For heavily sporulating cultures, such as Aspergillus and Penicillium species, or an actinomycete, only one tube of medium is necessary. Molds that rarely sporulate or do not sporulate heavily usually require two tubes. If the aqueous suspension is less than a MacFarland #4, the culture may not survive for long periods.

(2) Incubate slants at room temperature. Usually five to ten days is adequate for most mold cultures. Actinomycetes may require 14 to 20 days. H. capsulatum, B. dermatitidis, and C. immitis are cultured at room temperature and stored on slants in a sealed container at 4 to 10º C.

(3) Add approximately 5 mL of sterile distilled water to each slant.

(4) Sterilize one end of an approximately 8-inch-long, rounded-end, stainless steel, micro spatula in a Bunsen burner flame. Cool by inserting into a tube of sterile agar. Gently scrape all surface growth accumulated on the slant into the water, taking care not to remove any agar.

(5) Flame mouth of the culture tube and the sterile vial. aqueous suspension into the vial.

(6) Seal and store, as outlined in para a(4) above.

10-4. PLACING STOCK CULTURES UNDER OIL

If handled properly, stock cultures placed under oil will remain viable for at least ten years.

a. Sterilize approximately 100 mL of heavy mineral oil in a 250 mL flask by autoclaving at 15 psi for 45 minutes to one hour. Oil that appears cloudy after sterilization has absorbed water during autoclaving. Do not use.

b. When mold cultures have begun to sporulate (usually 5 to 10 days), or yeast and actinomycete cultures are well developed, on the agar slants, flame the mouths of the flask and the culture tube.

c. Gently pour sterile oil over entire agar slant. (Agar not covered by oil will act as a wick, enhancing water evaporation rate from the agar slant.)

d. Screw down the cap of the tube down and store in a dust-free area, at room temperature.

10-5. RECOVERING ORGANISMS FROM WATER STOCK

a. Place approximately 0.1 mL of sediment on the surface of a Sabouraud agar plate.

b. Streak inoculum across the agar as is done for a bacterial culture.

c. Incubate culture at room temperature, agar surface up.

d. When growth becomes obvious, examine all areas of plate for colonies characteristic of the fungus. Some pleomorphism will develop in the water stock culture and usually will appear as rapidly growing white colonies. To isolate typical colonies from the pleomorphic ones requires adequate surface area; hence, plates are used rather than agar slants.

e. Pick two or three typical colonies, inoculating each to an agar. Incubate at room temperature slant.

10-6. RECOVERING ORGANISMS FROM OIL SHOCK

Many molds and yeasts develop sterile aerial hyphae when placed under oil. It is essential to avoid these hyphae when recovering a culture from oil stock.

a. Flame the mouth of the stock culture tube and pour oil into a discard container. As oil is flowing into the container, take a sterile probe and dislodge any aerial mycelium present in the culture. Allow it to flow out with the oil. The portion of the fungal colony to be subcultured will adhere to the surface of the agar slant.

b. When oil has been decanted, continue to hold the tube at an angle. With a probe, remove some of the fungal colony and transfer it to a Sabouraud agar plate.

c. If possible, break the inoculum into small portions with a probe and distribute over the agar surface.

d. Incubate cultures at room temperature, agar surface up.

e. Subculture several typical colonies to agar slants.

Continue with Exercises

EXERCISES, LESSON 10

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement or by writing the answer in the space provided.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Name three reasons for maintaining stock fungus cultures.

   _____

   _____

   _____

2. The purpose of growing "working" stock cultures is to:

   a. Maintain readily available stock cultures.

   b. Monitor stock cultures for pleomorphism.

   c. Alternate types of media for the culture.

   d. Combine more than one organism in a stock culture.

3. What two media are used to maintain "working" stock culture of Microsporum gypseum*?*

   _____

   _____

4. Two methods for long-term maintenance of stock cultures are:

   _____

   _____

5. How long can stock cultures placed in sterile distilled water be expected to remain viable?

   a. 1 month.

   b. 1 year.

   c. 5 years.

   d. 10 years.

6. Organisms easily stored in sterile, distilled water include:

   _____

   _____

7. To maintain stock cultures under oil, sterile heavy mineral oil is poured over the agar slant:

   a. Immediately after it has been inoculated.

   b. So that the entire agar slant is covered.

   c. To a depth of 1/2 inch.

   d. After inoculation, and periodically thereafter.

8. To recover organisms from water stock:

   a. Streak 0.1 mL sediment on Sabouraud agar.

   b. Pour 0.1 mL supernatant water on Sabouraud agar.

   c. Inoculate typical colonies on a second Sabouraud plate.

   d. Incubate Sabouraud plate, reverse side facing up.

9. To recover organisms from oil stock, it is essential to:

   a. Inoculate a Sabouraud plate with an aliquot of overlaying oil.

   b. Avoid hyphae that have developed under oil.

   c. Inoculate portions of colony and oil on separate plates.

   d. Suspend portions of culture in sterile water for inoculation.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 10

1. Quality control of media.
   Reference for identification of unknown isolates.
   Training of new personnel. (para 10-1a).

2. a (para 10-2a)

3. Potato dextrose agar
   Dilute Sabouraud dextrose agar. (table 10-1).

4. Placing in sterile, distilled water
   Placing in sterile, mineral oil. (paras 10-3 and10-4)

5. c (para 10-3a(4))

6 Yeast
   Aerobic actinomycetes. (para 10-3)

7 b (para 10-4c)

8 a (para 10-5a)

9. b (para 10-6)

End of Lesson 10

LESSON ASSIGNMENT

LESSON 11	Commercial Kits
TEXT ASSIGNMENT	Paragraphs 11-1 through 11-4.
TASK OBJECTIVES	After completing this lesson, you should be able to select the statement that correctly describes the use of the API* 20C system for yeast identification. 11-2. Select the statement that correctly describes the use of the Minitek™ Yeast Identification system for yeast identification. 11-3. Select the statement that correctly describes the use of the Uni-Yeast-Tek™ system for yeast identification. 11-4. Select the statement that correctly describes the use of the Yeast-IDENT™ system for yeast identification.
SUGGESTION	After reading and studying the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.

LESSON 11

COMMERCIAL KITS

11-1. API* 20C IDENTIFICATION SYSTEM

a. **Purpose**. The API* 20C system is a ready-to-use micromethod, applying 19 assimilation tests to the identification of most clinically significant yeasts and yeast-like organisms. Biochemical reactions are complete after 72 hours of incubation at 30º C.

b. **Principle**. The API* 20C system consists of a series of cupules containing dehydrated substrates for assimilation reactions. These substrates are reconstituted by adding a yeast suspension in the API* Basal Medium to the cupules. Strips are incubated at 30º C. and read at 24-, 48-, and 72-hour intervals.

(1) Although assimilation tests form the basis for yeast identification, other information may be required. Microscopic morphology, such as germ tube production, presence *of* blastoconidia, arthroconidia, chlamydospores, pseudohyphae, true hyphae and capsule formation, should also be included before a final identification is made.

(2) Reactive substrates include glucose, glycerol, 2-keto-D-gluconate, L-arabinose, xylose, adonitol, xylitol, galactose, inositol, sorbitol, methyl-D-glucoside, N-acetyl-D-glucosamine, cellobiose, lactose, maltose, sucrose, trehalose, melezitose, and raffinose.

c. **Storage/Inspection**. The API* 20C system should be stored at 2º to 8º C. Strips can be removed from the pouch as needed. If the pouch is resealed properly, additional strips can be stored at 2º to 8º C. for up to 26 weeks or until the expiration date, whichever is first.

d. **Specimen Collection/Handling**. Specimens should be transported to the laboratory immediately after collection and processed as soon as possible after being received. For routine, primary isolations, most laboratories use Sabouraud dextrose agar, Sabouraud dextrose agar with antibiotics, and blood agar.

e. **Materials Required**.

(1) Materials provided with the API* 20C system include:

(a) API* 20C microcupule strips.

(b) Ampules of API* 20C Basal Medium.

(c) Incubation trays.

(d) Incubation covers.

(e) Report sheet.

(f) Package insert.

(g) Identification chart.

(2) Additional materials required:

(a) Sabouraud dextrose agar plates or slants.

(b) Sterile, wooden applicator sticks.

(c) Sterile Pasteur pipettes, 5 mL.

(d) 50 mL plastic squeeze bottle.

(e) Boiling chips.

(f) Safety goggles.

(g) Beaker.

(h) Marking pen.

(i) Incubator (30º C).

(j) Refrigerator (2º to 8º C).

(k) Water bath (50º C).

(l) Burner (Bunsen or Merker) or hot plate.

(m) Inoculating loop.

(n) Water.

(o) Sabouraud dextrose agar plates.

(p) API* 20C Code Book (optional).

f. **Procedure**.

(1) Prepare incubation tray by adding 10 mL of water. Water ensures adequate humidity.

(2) Place API* 20C strip in tray.

(3) Boil ampules of basal media, after allowing them to come to room temperature.

(4) Put ampules in water bath until temperature cools to 50º C.

(5) Prepare a yeast suspension in the ampule of basal media. Suspension should be prepared from a fresh culture of yeast grown for 48 to 72 hours on Sabouraud's dextrose agar. DO NOT USE SABOURAUD'S DEXTROSE AGAR WITH ANTIBIOTICS. Standardize suspension to slightly less than 1+ on a Wickerham card. Directions are included in the product insert.

(6) Fill each cupule with the yeast suspension.

(7) Incubate at 30º C. for 72 hours.

(8) Read and record reactions at 24, 48, and 72 hours.

(9) The "0" cupule serves as a negative control for the assimilation patterns. Cupules showing turbidity significantly heavier than the 0 cupule are positive. Positive reactions should be compared to turbidity in the GLU cupule that serves as a positive growth control.

(10) Identification can be made by using the differential charts included in each product insert or through generation of a code number and use of the API* 20C Analytical Profile Index.

g. **Quality Control**. Standard stock cultures from the American Type Culture Collection are recommended for individuals who do their own quality control.

(1) Cyptococcus laurentii ATCC 18803. See table 11-1.

(2) Trichosporon capitatum ATCC 10663. See table 11-1.

	EXPECTED REACTIONS	
	#1	#2
0	___=___	___=___
GLU	___+___	___+___
GLY	___=___	___+___
2KG	___+___	___=___
ARA	___+___	___=___
XYL	___+___	___=___
ADO	___+___	___=___
XLT	___+___	___=___
GAL	___+___	___=___
INO	___+___	___=___
SOR	___+___	___=___
MDG	___+___	___=___
NAG	___+___	___=___
CEL	___+___	___=___
LAC	___+___	___=___
MAL	___+___	___=___
SAC	___+___	___=___
TRE	__+/=__	___=___
MLZ	___+___	___=___
RAF	___+___	___=___

Table 11-1. Reactions.

11-2. MINITEK™ YEAST CARBON ASSIMILATION TEST

a. **Purpose**. The Minitek™ Yeast Carbon Assimilation Agar and the Minitek™ Yeast Set are designed as an aid in differentiating clinically significant yeasts. This differentiation is based on carbon assimilation patterns. The test does not allow identification to species level of all yeasts without assistance of additional tests.

b. **Principle**. A suspension of the test organism made in liquefied Minitek^{tm} Carbon Assimilation Agar is poured into a 150 mm petri dish and allowed to solidify. Selected Minitek™ substrate discs impregnated with specific carbohydrates are placed on the agar surface. The dish is then incubated and observed for growth around the discs. Growth indicates the organism is able to assimilate (use as a sole carbon source) that carbohydrate. No growth would indicate that the carbohydrate cannot be assimilated. The pattern developed by an individual organism, in conjunction with other associated data, forms the basis for the identification.

c. **Storage/Inspection**.

(1) Minitek™ Yeast Carbon Assimilation Agar: store unopened tubes at 2º to 30º C. Do not use if medium appears very turbid. A slight turbidity will not affect performance. Do not use the Yeast Carbon Assimilation Agar after the expiration date.

(2) Minitek™ Yeast Set: store at 2º to 8º C. After opening, keep refrigerated and desiccated when not in use. High moisture conditions may affect disc performance. Do not use discs after expiration date. Disc performance should be checked using known stock cultures. See Quality Control.

d. **Specimen Collection/Handling**. Clinical specimens must be processed to obtain isolated (separate) colonies on Sabouraud dextrose agar (SDA) or blood agar. The organism must be presumptively identified as a yeast by staining and any other appropriate tests. As with any microbiological specimen, strict aseptic techniques must be observed throughout processing.

e. **Materials Required**.

(1) Minitek™ Yeast Carbon Assimilation Agar: one tube per organism to be tested.

(2) Minitek™ Yeast Set: consisting of 12 cartridges of carbohydrate impregnated discs, 50 discs per cartridge. One set per 50 tests to be performed.

(3) Additional materials: 150 mm petri dishes, incubator at 30º C, inoculating loops or sterile polyester swabs, Sabouraud Dextrose Agar slants, sterile forceps, 2 water baths (one at 47º to 48º C, the other at 100º C), and tubes containing four mL of sterile water.

f. **Methodology**.

(1) The organism to be tested must be subcultured to an SDA slant. Incubate at 30º C. for 24 to 48 hours until good growth is obtained.

(2) Liquefy the Yeast Carbon Assimilation Agar by placing the tube in the boiling water bath just prior to use. When the agar is completely molten, place the tube in the 47 to 48º C. water bath. Another method used to melt the agar is to place it in the autoclave for one minute, then place the tubes in a 47º to 48º C. water bath until needed.

(3) Appropriately label one petri dish (150 mm) for each organism to be tested.

(4) Prepare a heavy suspension of the organism in a 4-mL tube of sterile water. The suspension should be equivalent to a No.5 MacFarland turbidity standard. The organism may be scraped off the SDA slant with a sterile inoculating loop or polyester swab. Thoroughly suspend the organism by mechanical mixing or shaking.

(5) Add the entire four mL of yeast suspension to one tube of molten agar (47º to 48º C.) Mix by inverting.

(6) Pour the entire 24 mL of inoculated medium into an appropriately labeled petri dish. Swirl gently to spread the agar over the entire dish. Allow to solidify at room temperature.

(7) Place one of each of the 12 carbohydrate discs on the agar surface. A 12 place self-tamping disc dispenser may be used for simplicity and easier spacing of the discs. Dextrose, maltose, and sucrose should be placed sequentially; other discs may be placed randomly. For ease of interpretation, however, the following pattern is recommended.

1. Dextrose without nitrate
2. Maltose
3. Sucrose
4. Lactose
5. Galactose
6. Melibiose
7. Cellobiose
8. Inositol
9. Xylose
10. Raffinose
11. Trehalose
12. Dulcitol

NOTE: If a self-tamping dispenser is not used, gently tamp the discs with sterile forceps.

(8) Incubate the plates at 30º C. for 24 to 48 hours.

g. **Interpretation**.

(1) Readings may be made at 24 hours, but the full 48-hour incubation time may be required for some organisms and/or carbohydrates.

(2) The presence of growth around a substrate disc is considered a positive assimilation reaction. The size of the zone of growth is dependent on the organism being tested and the particular carbohydrate. Therefore, a comparison should be made between growth around the disc, and growth in the surrounding agar. No growth around the disc should be interpreted as a negative test. The discs contain a pH indicator (phenol red) that may diffuse into the medium. If this happens, a yellow color will appear. It will not affect test reactions and should be disregarded.

(3) After all 12 reactions have been interpreted, identification may be made in one of two ways.

(a) Using the assimilation pattern obtained, consult table 11-2. Compare results to characteristics listed on a "best fit" basis to obtain identification.

(b) Consult the Minitek™ Yeast Code Book for instructions for interpreting results by "constructing" a numerical code that can be located in the book.

h. **Quality Control**. Stock cultures with known reactions should be tested for familiarization with the procedures/system, as well as to ensure appropriate techniques and interpretations are followed. Recommended Q. C. organisms are Candida albicans, and Cryptococcus laurentii.

11-3. UNI-YEAST-TEK™ YEAST IDENTIFICATION SYSTEM

a. **Purpose**. The Uni-Yeast-Tek™ plate is a rapid, simple method for presumptive identification of the most frequently identified yeasts isolated from clinical specimens.

b. **Principle**. The Uni-Yeast-Tek™ plate is composed of eleven separate peripheral wells, each containing a solid medium. The media will test for urease, nitrate, and various carbohydrate fermentations. A central well on the wheel contains corn meal tween agar used to observe the yeast's production of characteristic morphological structures, such as blastoconidia, arthroconidia, chlamydospores, and so forth.

c. **Storage/Inspection**. Uni-Yeast-Tektm™ plates should be stored at 2º to 8º C. and used prior to the expiration date stamped on each plate. Each plate should be checked prior to use to ensure it is still usable. The urea well should be straw colored, carbohydrate wells purple, nitrate wells yellow, and corn meal tween well white to cream.

NOTE: Particles may appear in the nitrate well while the plate is refrigerated. These particles are precipitated dye that redissolve and disappear when the plate is returned to room temperature. These particles do not mean the plate is contaminated.

d. **Specimen Collection/Handling**. No special procedures need to be followed for collection and handling of specimens. Routinely established methods discussed in this publication should be used.

e. **Materials Required.** Microscope, incubators (35/37 and 30º C.), Bunsen burner, sterile Pasteur pipettes, pipette bulbs, sterile, glass microscope slides, sterile cover slips (22 mm square), inoculating needle and loop, forceps, alcohol for sterilization, test tube rack, marking pen, Uni-Yeast-Tek™ plate, 12 x 75 mm tube containing 0.3 to 0.5 mL of sterile serum for germ tube test, and a 12 x 75 mm test tube containing 2.0 mL of sterile water.

f. **Methodology**.

(1) The yeast being tested must be in pure culture on an SDA slant, 18 to 24 hours old.

(2) Using the needle, inoculate the germ tube test from the yeast culture. Incubate the inoculated tube at 37º C. for 2 to 4 hours. After incubation, withdraw a drop of serum from the germ tube test tube and place it on a glass slide, cover slip, and examine for presence of germ tubes. If germ tubes are present, perform step #3 below. If germ tubes are absent, skip step #3 and proceed to step #4.

(3) Only two organisms should give positive tests for germ tube formation, Candida albicans and Candida stellatoidea. Inoculation of a tube of sucrose assimilation media will provide the necessary differentiation since Candida albicans has the ability to assimilate sucrose, while Candida stellatoidea has not. The Uni-Yeast-Tek™ plate is not needed for germ tube-positive organisms.

(4) For germ tube-negative organisms, remove seal from a Uni-Yeast-Tek™ plate and label lid and plate to correspond to the specimen being examined.

(5) Using a flamed, sterile, inoculating needle, inoculate a tube containing 2.0 mL of sterile water with growth from the SDA slant. Suspension should be slightly turbid. Reflame needle.

(6) Using a sterile Pasteur pipette, withdraw approximately three-fourths of the liquid in the tube. Add one drop of suspension to each of the eleven wells on the Uni-Yeast-Tektm™ plate.

NOTE: To avoid missing any well or inoculating any more than once, begin with the same well each time. Begin with the urea well and inoculate around the plate until the urea well is reached again. Dispose of pipette and excess suspension in suitable disinfectant.

(7) Flame and cool the inoculating needle. Pick a small amount of colony on the Sabouraud agar and "scratch" center of the corn meal tween well in the center of the plate. Streak perpendicular to initial scratch two or three times. Reflame needle.

(8) Using sterile forceps, pick up a sterile cover slip (cover slip may be sterilized by flaming and cooling) and place it over scratched area in center well. Cover plate with correctly labeled lid and incubate at 30º C.

(9) Examine after 24 hours and daily for a maximum period of six days. Interpret biochemical results according to table 11-2.

TEST	POSITIVE	NEGATIVE
UREA	pink	straw
SUCROSE	yellow	purple
LACTOSE	yellow	purple
MALTOSE	yellow	purple
RAFFINOSE	yellow	purple
CELLOBIOSE	yellow	purple
SOLUBLE STARCH	yellow	purple
TREHALOSE	yellow	purple
SUGAR CONTROL	always purple	
NITRATE	blue or green	yellow
NITRATE CONTROL	always yellow	
CORN MEAL (center well)	read microscopically	

Table 11-2. Interpretation of Uni-Yeast-Tek™ plates.

(10) After interpreting biochemical results:

(a) Develop a biogram number from table 11-3 and use the Flow* Yeast Computer Code Book to identify the organism. Total the numbers under the positive tests within each group of three tests. Use this number to identify the organism in the Flow* Yeast Computer Code Book. Perform and interpret additional tests as indicated in the code book.

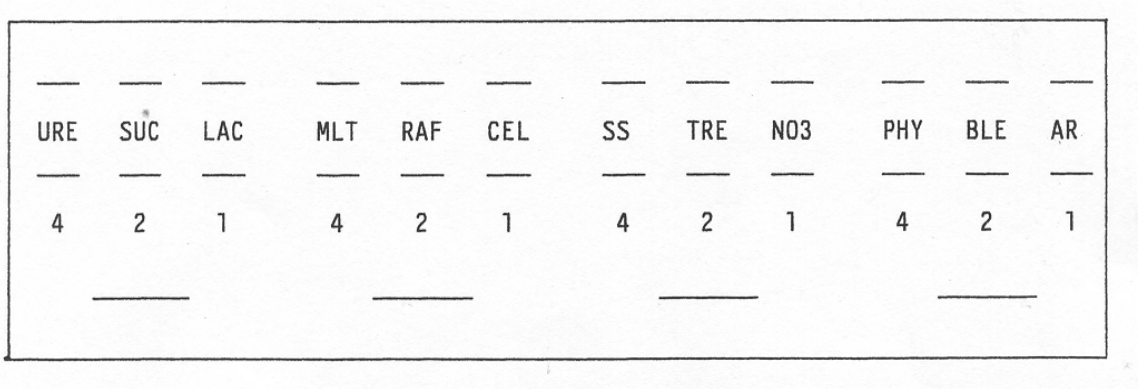


Table 11-3. Biogram number chart.

(b) Use the chart in Table 11-4 to determine the organism identification.

(11) After the interpretation and identification are complete, autoclave the plate for 15 minutes at 15 psi before discarding.

YEAST WHELL I.D. KEY

TEST	RESULTS		IDENTIFICATION	GO TO
1 GERM TUBE	Positive			3 - Sucrose
		Negative		2 - Urease
2 UREASE	Positive			10 - Corn Meal Tween
		Negative		4 - Lactose
3 SUCROSE	Positive		*C. albicans*	
		Negative	*C. stellatoidea*	
4 LACTOSE	Positive		*C. pseudotropicalis*	
		Negative		5 - Maltose
5 MALTOSE	Positive			7 - Raffinose
		Negative		6 - Trehalose
6 TREHALOSE	Positive		*T. glabrata*	
		Negative	*C. Krusei* or *Geotrichum* spp	10 - Corn Meal Tween
7 RAFFINOSE	Positive			8 - Cellobiose
		Negative		9 - Soluble Starch
8 CELLOBIOSE	Positive		*C. guilliermondii*	
		Negative	*C. cervisiae*	
9 SOLUBLE STARCH	Positive		*C. tropicalis*	
		Negative	*C. parapsilosis*	
10 CORN MEAL TWEEN	True hyphae Pseudohyphae Blastoconidia & Arthroconidia		*Trichosporon* spp.	11 - Nitrate
	Non-pigmented Colony Blastoconidia Only		*Cryptococcus*spp.	12 - Nitrate
	Pigmented Colony Blastoconidia Rare pseudohyphae		*Rhodotorula* spp.	15 - Nitrate
	Pseudohyphae & Blastoconidia		*C. krusei*	
	True hyphae & Arthroconidia		*Geotrichum* spp.	
11 NITRATE	Positive		*Tr. pullulans*	
		Negative	*Tr. cutaneum*	
12 NITRATE	Positive			13 - Sucrose
		Negative		14 - Lactose
13 SUCROSE	Positive		*Cr. albidus*	
		Negative	*Cr. terreus*	
14 LACTOSE	Positive		*Cr. laurentii*	
		Negative	*Cr. neoformans*	
15 NITRATE	Positive		Rh. glutinis	
		Negative	Rh. rubra	

Table 11-4. Organism identification (continued).

	UREA	SUCROSE	LACTOSE	MALTOSE	RAFFINOSE	CELLOBIOSE	SOLUBLE STARCH	TREHALOSE	SUGAR CONTROL	NITRATE	NITRATE CONTROL	PSEUDOHYPHAE/HYPHAE	BLASTOCONIDIA	ARTHROCONIDIA	PIGMENT RED	CHLAMYDOSPORES	GERM TUBE
Candida albicans	-	+	-	+	-	-	+	+	-	-	-	+	+	-	-	+	+
Candida stellatoidea	-	-	-	+	-	-	+	+	-	-	-	+	+	-	-	-	+
Candida parapsilosis	-	+	-	+	-	-	-	+	-	-	-	+	+	-	-	-	-
Candida tropicalis	-	+	-	+	-	+	+	+	-	-	-	+	+	-	-	-	-
Candida pseudotropicalis	-	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-
Candida guilliermondii	-	+	-	+	+	+	V	+	-	-	-	+	+	-	-	-	-
Candida krusei	V	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Geotrichum spp.	V	-	-	-	-	-	V	-	-	-	-	+	-	+	-	-	-
Saccharomyces cerevisiae	-	+	-	+	+	-	-	V	-	-	-	+	+	-	-	-	-
Torulopsis glabrata	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
Rhodotorula rubra	+	+	-	+	+	V	V	+	-	-	-	-	+	-	+	-	-
Rhodotorula glutinis	+	+	-	+	+	+	-	+	-	+	-	-	+	-	+	-	-
Cryptococcus albidus	+	+	+	+	+	+	V	+	-	+	-	-	+	-	-	-	-
Cryptococcus neoformans	+	+	-	+	+	+	V	+	-	-	-	-	+	-	-	-	-
Cryptococcus laurentii	+	+	+	+	+	+	V	+	-	-	-	-	+	-	-	-	-
Cryptococcus terreus	+	-	+	V	-	+	V	+	-	+	-	-	+	-	-	-	-
Trichosporon cutaneum	+	+	+	+	V	+	V	V	-	-	-	+	+	+	-	-	-
Trichosporon pullulans	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	-

Table 11-4. Organism identification (concluded).

g. **Quality Control**. Table 11-5 indicates results that should be obtained using the control stock cultures indicated.

	ATCC 13243 *C. krusei*	ATCC 10656 *C. albidus*	ATCC 14053 *C. albicans*	CDC 42-74-002371 *C. neoformans*
GERM TUBE	-	-	-	-
UREA	- S	+ Pi	- S	+ S
SUCROSE	- S	+ Y	- S	- S
LACTOSE	- P	L Y	+ Y	- P
MALTOSE	- P	+ Y	+ Y	+ Y
RAFFINOSE	- P	+ Y	- P	+ Y
CELLOBIOSE	- P	+ Y	- P	+ Y
SOLUBLE STARCH	- P	+ Y	+ Y	+ Y
TREHALOSE	- P	+ Y	+ Y	L Y
SUGAR CONTROL	- P	- P	- P	- P
NITRATE	- Y	+ G	- Y	- Y
NITRATE CONTROL	- Y	- Y	- Y	- Y
CORN MEAL TWEEN	b & p	b	c, p & b	b

b = blastoconidia C = chlamydospores p = pseudohyphae

KEY Pi = Pink Y = Yellow S = Straw P = Purple
L = Latent G = Green/Blue - = Negative + = Positive

Table 11-5. Yeast wheel quality control.

11-4. API* YEAST-IDENT™ SYSTEM

a. **Purpose**. The API* Yeast-IDENT™ system is a rapid, standardized, sensitive micromethod of identifying clinically significant yeasts and yeast-like organisms, using miniaturized, conventional, and chromogenic tests. The tests are read after four hours of incubation at 35º to 37º C.

b. **Principle**. The Yeast-IDENT™ system consists of 20 microcupules containing dehydrated substrates. Adding a suspension of the yeast in distilled water dehydrates the substrates. The microcupule forms a supporting chamber that is especially designed for enzymatic tests. The URE microcupule contains phenol red as an indicator that turns pink or red if positive. The PHS, BDF, BDG, NGL, and ADG microcupules rely on a color change to straw or yellow, produced when p-nitrophenol is released. The BDX microcupule turns straw or yellow, when o-nitrophenol is produced. The PNA microcupule also turns yellow when p-nitroaniline is released. The INA microcupule produces a dark blue precipitate, when indoxyl is the end product. The GLY, PRO, TRP, HPR, ILU, VAL, LUG, HIS, CYS, TYR, and GLG microcupules require the addition of Cinnamaldehyde reagent to detect the liberated B-naphthlyamine which turns pink, red, or purple.

c. **Storage/Inspection**. The Yeast-IDENT™ system should be stored at 2º to 8º C. After opening the pouch containing the strips, the unused strips can be resealed by taping. Then can be stored up to three months or until the expiration date. The desiccant packet must be kept inside when resealing unused strips.

d. **Specimen Collection/Handling**. Specimens should be transported to the laboratory immediately after collection and processed as soon as possible after being received. Most laboratories use Sabouraud dextrose agar along with blood agar and Sabouraud dextrose agar with antibiotics for routine primary isolation.

e. **Materials Required**.

(1) Materials provided with the Yeast-IDENT™ system.

(a) Yeast-IDENT^{tm} strips.

(b) Incubation trays.

(c) Incubation covers.

(d) Report sheet.

(e) Package insert.

(2) Additional materials required.

(a) Sabouraud Dextrose Agar Plates or Slants.

(b) Sterile wooden applicator sticks or cotton swabs.

(c) Sterile Pasteur pipettes, 5 mL or 100 µl pipettes.

(d) Sterile distilled water (pH 5.5 to 7.0).

(e) Sterile plastic or pre-washed tubes.

(f) Test tube rack.

(g) Cinnamaldehyde reagent.

(h) Marking pen.

(i) Inoculating loop.

(j) Burner (Bunsen or Merker) or hot plate.

(k) Refrigerator (2º -8º C).

(l) Incubator (35º -37º C).

(m) Incubator (25º -30º C).

f. **Procedure**.

(1) Set up incubation tray and lid.

(2) Record patient's specimen number on elongated tray flap.

(3) Remove strips from pouch and place one strip in each incubation tray.

(4) Add 3 mLs distilled water (pH 5.5 to 7.0) to a sterile plastic or prewashed, glass test tube.

(5) Remove test organisms from a 48 to 72 hour culture on a Sabouraud Dextrose Agar plate or slant, using a sterile applicator stick or swab to pick sufficient colonies to make a final turbidity of less than 3+ on a Wickerham card. (See package insert for complete instructions and Wickerham card.)

(6) Withdraw the yeast suspension from the tube using a sterile 5 mL Pasteur pipette.

(7) Fill each microcupule with 2-3 drops (approximately 100 µl) of the yeast suspension.

(8) Use the excess yeast suspension to inoculate a SDA for purity check, and/or additional biochemical testing. Incubate the plate for 48 to 72 hours at 25º to 30º C.

(9) After inoculation, place the plastic lid on the tray and incubate the strip for 4 hours at 35º to 37º C. in a humidified non-CO_2 incubator.

(10) Strips should be read as soon as possible after 4 hours incubation at 35º to 37º C. Do not over incubate. Strips can be held at room temperature for up to a maximum of 30 minutes after incubation.

(11) Allow the cinnamaldehyde reagent to come to room temperature and add one drop to the remaining microcupules. Record reactions three minutes after addition of the reagent. Color reactions usually begins within 30 seconds. Color development occurring after three minutes should NOT be interpreted as positive.

(12) After four hours of incubation, record the results for all tests not requiring the addition of reagent (URE through INA). Positive reactions listed in the principle section.

(13) After all reactions have been recorded on the report sheet and a satisfactory identification has been made, the entire test unit should be autoclaved, incinerated, or immersed in a germicide prior to disposal.

(14) Although the Yeast-IDENT™ system uses the 20 biochemical tests contained in the microcupules, other information must also be used. Specimen source, colony morphology, microscopic characteristics, temperature tolerance tests, and additional biochemicals should be used for final identification of clinically significant yeasts and yeast-like organisms.

g. **Quality Control**. For those who wish to do their own quality control, standard stock cultures from the American Type Culture Collection are recommended. See table 11-6 for recommended quality control organisms and expected reactions.

RECOMMENDED ORGANISMS

1. *Candida albicans* ATCC 14053
2. *Candida tropicalis* ATCC 13803
3. *Cryptococcus albidus* ATCC 60109
4. *Cryptococcus neoformans* ATCC 32045
5. *Rhodotorula glutinis* ATCC 32765
6. *Rhodotorula minuta* ATCC 60108

EXPECTED REACTIONS (Keyed to organisms listed above)

	#1	#2	#3	#4	#5	#6
URE	=	=	+	+	+	+
PHS	v	v	+	v	+	=
BDF	=	=	=	=	=	+
BDG	=	v	+	v	v	+
NGL	+	=	=	=	=	=
ADG	v	+	v	=	v	v
BDX	=	=	+	=	v	=
PNA	+	=	+	=	+	v
INA	v	v	+	=	+	+
GLY	v	+	+	=	+	+
PRO	+	=	+	=	+	v
TRP	v	+	+	=	+	v
HPR	+	=	v	=	+	v
ILU	=	v	v	=	+	v
VAL	v	v	+	=	+	+
LUG	=	+	+	=	+	+
HIS	v	+	+	=	+	+
CYS	v	v	+	=	v	v
TYR	=	v	+	=	+	v
GLG	v	+	+	=	v	+

Table 11-6. API Yeast-IDENT™ system quality control.

Continue with Exercises

EXERCISES, LESSON 11

INSTRUCTIONS: Answer the following exercises by marking the lettered responses that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided.

After you have completed all of the exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Strips for the API* 20C are incubated for:

   a. 4 hrs.

   b. 72 hrs.

   c. 4 days.

   d. 6 days.

2. API* 20C system should be stored at:

   a. 0º -4º C.

   b. 2º -8º C.

   c. 20º -30º C.

   d. 40º -60º C.

3. Which of the following represents a positive growth control factor in the API* 20C system?

   a. 0 cupule.

   b. ARA cupule.

   c. POS cupule.

   d. GLU cupule.

4. Growth of a yeast around Minitek™ substrate disc indicates that:

   a. Specific carbohydrate has been assimilated.

   b. Specific carbohydrate has not been assimilated.

   c. Nonsterile technique was used in inoculation.

   d. Positive identification of a yeast organism has occurred.

5. The medium used to process clinical specimens for the Minitek™ Carbon

   Assimilation Test is _____ .

6. Which of the following statements represents the quantity of test organism used in the Minitek™ Yeast Assimilation Test?

   a. One or two colonies suspended in 4 mL sterile water.

   b. A suspension in sterile water, equivalent to a No.5 MacFarland turbidity standard.

   c. A heavy suspension of test organism in test medium.

   d. The entire colonial growth resulting from a 48 hour incubation.

7. Prepared plates from the Minitek™ Yeast Assimilation tests are incubated at

   _____ for _____.

8. What organism can be used for quality control with the Minitek™ Yeast Assimilation test?

   a. Only Candida albicans.

   b. Only Cryptococcus laurentii.

   c. Candida albicans and Cryptococcus laurentii.

   d. Cryptococcus neoformans and Cryptococcus laurentii.

9. Media used in the Uni-Yeast-Tek™ plate test for urease, nitrate, various carbohydrate fermentation, and:

    a. Carbohydrate assimilation.

    b. Characteristic morphology.

    c. Macroscopic morphology.

    d. Inhibition of colonial growth.

10. If a refrigerated Uni-Yeast-Tek™ plate has particles in the nitrate well, the plate is contaminated and should be discarded.

    a. True.

    b. False.

11. The only organism that is positive for germ tube production and sucrose assimilation is:

    a. Candida albicans.

    b. Candida tropicalis.

    c. Candida stellatoidea.

    d. Candida krusei.

12. Inoculated Uni-Yeast-Tek™ plates are incubated for:

    a. 4 hrs.

    b. 72 hrs

    c. 4 days.

    d. 6 days.

13. Before reading the tests, a Yeast-IDENT™ strip is incubated for:

    a. 2 days.

    b. 3 days.

    c. 24 hours.

    d. 4 hours.

14. The reagent added to the Yeast-IDENT™ strip prior to reading specified color reactions is:

    a. Cinnamaldehyde.

    b. Ferric chloride.

    c. Kovac's.

    d. Phenol red.

Check Your Answers on Next Page

SOLUTIONS TO EXERCISES, LESSON 11

1. b (para 11-1a)

2. b (para 11-1c)

3. d (para 11-1f(10)

4. a (para 11-2b)

5. Sabouraud dextrose agars or blood agar. (para 11-2d)

6. b (para 11-2f(4))

7. 30º C.
   24 to 48 hours. (para 11-2f(8))

8. c (para 11-2h)

9. b (para 11-3b)

10. b (para 11-3c)

11. a (para 11-3f(3))

12. d (para 11-3f(9))

13. d (para 11-4a)

14. a (para 11-4b)

End of Lesson 11

APPENDIX

GLOSSARY OF TERMS

A

Aerial mycelium: mycelium that grows above the medium surface (into the air) and provides texture to the colony.

Anamorph: the asexual or somatic reproductive form of an organism.

Annellate: having annellations.

Annellation: a ring of outer cell wall material remaining at the apex of an annellide that resulted from the release of an annelloconidium.

Annellide: a conidiogenous cell that produces an annelloconidium.

Annelloconidium: a conidium produced from an annellide.

Anthropophilic: man-loving. A term usually applied to fungi that usually infects only humans.

Apex: the tip.

Apothecium: an open or expanded fruiting body whose asci are contained on its exposed surface.

Arthroconidium: a conidium that results from the fragmentation of a true hypha into separate cells.

Ascocarp: a structure, characteristic of the class Ascomycetes, in which asci are formed. There are three types: perithecium, cleistothecium and apothecium.

Ascospore: a sexual spore, characteristic of the class Ascomycetes. It is produced, following the union of two nuclei, in a sac-like structure called an ascus.

Aseptate: the absence of cross walls in a hyphal filament or spore.

Assimilation: the ability to use a carbon or nitrogen source for growth, with oxygen being the final electron acceptor. Assimilation is read as the presence or the absence of growth.

B

Basidiomycetes: a taxonomic class of fungi characterized by the production of sexual spores on a club-shaped structure known as a basidium. Some examples are mushrooms, toadstools, plant rusts and smuts, as well as the pathogen to man, Cryptococcus neoformans.

Basidiospore: a sexual spore, characteristic of the class Basidiomycetes, produced, following the sexual union of two nuclei, on a specialized club-shaped structure known as a basidium.

Basidium: a cellon which basidiospores are formed as a result of karyogamy and meiosis. Basidia are characteristic of the class Basidiomycetes.

Blastoconidium: a conidium produced by a budding process along the mycelium, from a previous conidium or from a vegetative cell (yeast).

Budding: an asexual reproductive process characteristic of unicellular fungi involving the formation of lateral outgrowths from the parent cell, which, after maturing, pinch off to form a new cell.

C

Candidiasis (candidosis): a human or lower animal infection caused by a member of the genus Candida.

Cell: any unit, conidium or spore that is separated from its neighbors, usually by a wall or septum.

Cell wall: the wall enclosing the cytoplasm of a cell. In fungi it consists of a complex network of fibrils, the spaces of which are filled with polymers.

Channel: a secondarily induced pore in the outer wall of a conidiogenous cell that is usually thick and darkly pigmented.

Chitin: a linear molecule that is a major component of most fungal cell walls.

Chlamydospore: a cell of a septate hypha or pseudohyphae that rounds up and develops a cell wall thicker than the walls of other cells within the hypha. It may be located within the hypha (intercalary), at the end of the hypha (terminal), or on a short lateral branch of the hypha.

Chromoblastomycosis (chromomycosis): a mycotic infection of cutaneous and subcutaneous tissues characterized by the development in tissue of dematiaceous sclerotic bodies.

Cladosporium-type copulation: a type of spore formation, characteristic of the genera Cladosporium and Fonsecaea, in which oval conidia are produced in branched chains by various length conidiophores.

Clavate: club-shaped.

Cleistothecium: a structure, usually spherical, in which asci are formed. The asci are enclosed within the cleistothecium and are released when it ruptures.

Coccidioidomycosis: a human or lower animal infection caused by Coccidioides immitis.

Collarette: a small collar; usually applied to cell wall remnants at the tip of a phialide resulting from rupture of the tip during release of initial phialoconidium.

Columella: the sterile, dome-shaped apex of a sporangiophore characteristic of the class Zygomycetes.

Conidiogenous: giving rise to conidia.

Conidiogenous cell: a cell that produces conidia.

Conidiophore: a specialized hypha on which conidia are formed.

Conidium (pl. conidia): a single asexual cell that may produce a new conidium, true hypha or pseudohyphae. It may be unicellular or multicellular, and may vary in size and shape.

Cryptococcosis: a human or lower animal infection caused by Cryptococcus neoformans.

Cutaneous: pertaining to the skin.

D

Dematiaceous: having conidia, spores and or hyphae that are brown to black in color because of the presence of melanin.

Denmatophyte: any fungus that is parasitic on skin, nails or hair of humans or animals.

Dermatophytosis: a disease caused by a dermatophyte.

Deuteromycetes: see "imperfect fungi."

Diphasic (Dimorphic): having two morphological forms. Commonly used to describe fungi that grow as a mold at room temperature and as either a yeast or spherule at 35°C.

E

Eccentric: away from the center.

Echinulate: covered with spines.

Ectothrix: a natural invasion of the hair by a dermatophyte that is characterized by arthrocondia outside, and mycelium inside, the hair shaft, and destruction of the hair cuticle.

Endogenous: originating from within.

Endospore: a spore produced within a spherule. Endospores are sporangiospores.

Endothrix: a natural invasion of the hair by a dermatophyte that is characterized by the development of arthrocondia only within the hair shaft.

En grappe: a term used to describe the formation of microconidia in clumps on the ends of hyphae. Usually used to refer to the genus Trichophyton.

En thyrse: a term used to describe the formation of microconidia along the sides of hyphae. Usually used when referring to the genus Trichophyton.

Epilation: removal of hair by the roots.

Eukaryotic: having a highly organized nucleus bounded by a nuclear membrane.

Eumycotic granule: a granule produced by a fungus.

Eumycotic mycetoma: a mycetoma caused by a true fungus.

Evulsed: extracted by force.

Exogenous: originating from without.

F

Fermentation: the ability of a fungus to use a carbon source for growth with organic compounds serving as the electron donor and acceptor.

Fluorescence: the property of emitting light of characteristic color upon exposure to filtered ultraviolet rays.

Fruiting Body: fungal structure bearing sexual spores.

Fungus: a eukaryotic, unicellular to filamentous organism that usually reproduces by both a sexual and asexual process. Fungi are usually slow growers and non-fastidious.

Fusiform: spindle-shaped, tapering towards the end.

G

Geniculate: bent like a knee.

Geophilic: term applied to fungi whose natural habitat is in soil.

Germ tube: a tube-like structure on a germinating cell that later develops into a hypha.

Glabrous: smooth, leather-like.

Gymnothecium: a fruiting body consisting of a loose network of mycelium through which aeciospores filter and are released following maturation.

H

Hilum: a depression or pit at the site in an organ where vessels and nerves enter.

Hyaline: colorless.

Hypha (pl. hyphae): a single filament of a fungus.

I

Imperfect fungi: those fungi that have not as yet demonstrated a sexual form of reproduction. This is characteristic of the class Deuteromycetes.

Intercalary: produced within two hyphal segments.

Intercellular: between cells.

Intracellular: within cells.

J - K

Keratin: a scleroprotein containing large amounts of sulfur, such as cystine.

L

Lipophilic: an organism that has an affinity for fat.

M

Macroconidium (pl. macroconidia): a large, multicellular conidium.

Meiosis: a special method of cell division, occurring in maturation of sex cells, by which each daughter cell receives half the number of chromosomes characteristic of the somatic cells of the species.

Merosporangium: Sporangium containing sporangiospores aligned in a row.

Metula (pl. metulae): a sterile branch below the phialides of Aspergillus and Penicillium spp.

Microconidium (pl. microconidia): a small, single-celled conidium.

Mitosis: a process of nuclear division by which the two daughter nuclei receive a complement of chromosomes identical to that of the parent.

Mold: a multicellular thread-like fungus.

Monilia: a former name for the genus now called Candida.

Monophasic (monomorphic): having only one distinct form of growth (either a mold or a yeast).

Multiseptate: having several septa.

Muriform: possessing both horizontal and vertical septations.

Mycelium: a mass of hyphae making up the thallus (body) of a fungus.

Mycology: the branch of science that studies fungi and their biology.

Mycosis: an infection or disease caused by a fungus.

Mycotic: pertaining to a fungal disease.

N - O

Onychomycosis: a general term for a fungal infection of the nails.

Oospore: a thick-walled sexual spore produced through fusion of two unlike gametes that is characteristic of some fungi in the class Zygomycetes.

P

Perfect fungi: those fungi that demonstrate both sexual and asexual forms of reproduction. A perfect state refers to the sexual form of reproduction.

Perithecium: a special round, oval or beaked structure in which asci are formed, and which possesses a natural opening allowing release of the asci.

Phialide: a conidiogenous cell, which produces a phialoconidium.

Phialoconidium: a conidium produced from a phialide.

Phialophora-type sporulation: a type of asexual sporulation in which phialoconidia are produced from vase-shaped phialides having a flared collarette. Characteristic of Phialophora verrucosa and the genus Fonsecaea.

Piedra: an infection of the hair shaft characterized by the presence of irregular nodules.

Pleomorphism: an irreversible degenerative change in a fungus that converts the colony into one that is completely sterile. Characteristic and diagnostic cells are degenerated or lost.

Polymorphic: having more than one morphological form which are not temperature dependent and which exist in culture simultaneously.

Poroconidium: a conidium produced through a minute pore or channel in the cell wall of a conidiophore or conidiogenous cell.

Propagule: an individual unit that can give rise to another organism.

Pseudohyphae: a chain of elongated blastoconidia that do not separate. Cells are usually constricted at the points of attachment.

Pyriform (piriform): ear-shaped.

Q - R

Racquet hyphae: vegetative hyphae showing terminal swelling of segments resembling a tennis racquet in shape.

Rhinocladiella sporulation: a type of sporulation in which oval conidia are formed along the sides of irregular club-shaped conidiophores.

Rhizoid: short branching hyphae that resemble a "root". Characteristic of some fungi in the class Zygomycetes.

Ringworm: a term used to designate a fungal infection caused by a dermatophyte. It originated from the ancient belief that infections were caused by a worm and from the fact that sometimes lesions are circular.

Rudimentary: primitive, poorly developed.

S

Saprophyte: an organism that uses dead organic matter as a source of food.

Sclerotic body: a cluster of thick-walled, dematiaceous, rounded cells. Sclerotic bodies are diagnostic of chromoblastomycosis.

Septate: the presence of cross-walls in either a hyphal element or a conidium.

Septum: a cross-wall in a hyphal filament or conidia formed in a centripetal manner from the cell wall.

Sexual reproduction: involving the fusion of two compatible nuclei.

Shield cell: a conidium of Cladosporium that has the general shape of a shield.

Sinus: an abnormal channel.

Spherule: a thick-walled, sporangium-like structure produced within tissue and in vitro by Coccidioides immitis.

Spirals: tightly coiled hyphae found in many fungi.

Sporangiophore: a specialized hypha bearing a sporangium.

Sporangium: an asexual sac-like structure that produces sporangiospores.

Spore: a unicellular unit derived by sexual or a sexual means which, if formed by asexual means, involves a cleavage process.

State: a phase or condition in the life cycle of a fungus.

Stratum corneum: the outer layer of the epidermis consisting of dead desquamating cells.

Subcutaneous: below the skin.

Superficial: near the surface.

Sympodial: a type of sporulation where blastoconidia are produced at the apex of an onidiophore. As a blastoconidia is produced on the apex, a new apex is developed behind and to the side of the previous apex. Ex. Sporothrix spp.

Systemic: affecting the body as a whole.

T

Teleomorph: the sexual form of an organism; also called the perfect state.

Thallus: the vegetative part of a fungus body consisting of undifferentiated cells.

Tinea (ringworm): a superficial fungal infection usually caused by dermatophyte. Ex. Tinea capitis (ringworm of the scalp).

Truncate: ending abruptly, cut-off sharply.

Tuberculate: having small, knob-like projections.

U

Uniserate: phialide forming directly on the vesicle (in reference to the genus Aspergillus).

V

Vegetative hyphae: hyphae that absorb nutrients needed for resting growth. This is what is recovered and seen on a direct mount.

Verticil: a whorl (cluster) of conidiophores or conidiogenous cells from a common site.

Vesicle: a swollen cell. Ex. the swollen apex of the conidiophore in Aspergillus spp.

W - X - Y

Yeast: a unicellular budding fungus.

Z

Zoophilic (animal-loving): a term applied to fungi that infect lower animals as well as humans.

Zygomycetes: a taxonomic class of fungi whose sexual reproduction is by means of oospores or zygospores. The asexual stage of reproduction usually consists of rarely septate hyphae and sporangia with sporangiospores.

Zygospore: a thick-walled sexual spore produced from fission *of* two similar gametangia. Characteristic *of* the class Zygomycetes.

End of Appendix